Evaluation Of *ASVERADO* For Its Anti-Neoplastic Activity Against 7,12- Dimethyl benz[a]anthracene (DMBA) Induced Mammary Gland Carcinoma In Female Wistar Rats

Dissertation submitted to

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In partial fulfilment for the award of the degree of

MASTER OF PHARMACY

IN

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By

Khyathi .D. Sanghvi

Register No: 261525004

UNDER THE GUIDANCE OF

Dr. P.MURALIDHARAN, M. Pharm., Ph.D

DEPARTMENT OF PHARMACOLOGY

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Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

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CERTIFICATE

This is to certify that Project entitled ***Evaluation Of ASVERADO For Its Anti-Neoplastic Activity Against 7,12- Dimethyl benz[a]anthracene (DMBA) Induced Mammary Gland Carcinoma In Female Wistar Rats*** submitted by Register No: 261525004 in partial fulfilment of the course for the award of the degree of **Master of Pharmacy in Pharmacology .** It was carried out at Department of Pharmacology in C.L. Baid Metha College of Pharmacy, Chennai-97 under my guidance during the academic year 2016-2017.

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Date:

(**Prof. Dr. GRACE RATHNAM)**

Place: Chennai-97

DECLARATION

Register No.261525004 , hereby declare that this dissertation entitled, **'*Evaluation Of ASVERADO For Its Anti-Neoplastic Activity Against 7,12-Dimethylbenz[a]anthracene (DMBA) Induced Mammary Gland Carcinoma In Female Wistar Rats'***, has been originally carried out by me under the guidance and supervision of **Prof. Dr.P.Muralidharan, M.Pharm,. PhD,** Head of the department of pharmacology, C.L. Baid Metha College of Pharmacy, Chennai-97 for the academic year 2016-2017. This work has not been submitted in any other degree at any other university.

Date: Register No. 261525004

Place: Chennai-97

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I am making this project not only for marks but to also increase my knowledge.

Thanks again to all who helped me.

INDEX

List of Abbreviations

α	Alpha
β	Beta
γ	Gamma
%	Percentage
&	And
μm	Micrometer
μg	Microgram
μl	Microlitre
ANOVA	Analysis of variance
ASVERADO	Ashwagandha ,Aloe vera, Avocado
B.C	Botanical classification
BRAC	BReast CAncer susceptibility gene
CAM	Complementary and alternative medicine
Cm	Centimeter
ºC	Degree Celsius
DES	Diethylstilbestrol
DMBA	7,12dimetylbenz[a]anthracene
DNA	Deoxy-ribo Nucleic Acid
DCIS	Ductal carcinoma in situ
EDTA	Ethylene Diamine Tetra Acetic

	acid
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
G	Gram
h, hr	Hour (s)
Hb	Hemoglobin
Ht	Hormone therapy
HRT	Hormone replacement therapy
IC50	Median inhibitory concentration
i.p	Intraperitoneal
IDC	Invasive ductal carcinoma
Kg	Kilogram
L	Litre(s)
MCV	Mean corpuscular volume
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCF-7	Michigan Cancer Foundation-7
Mg	Milligram
Min	Minute(s)
Ml	Millilitre(s)
mM	Millimole(s)
MRI	Magnetic resonance imaging
MTT	3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyltetrazolium bromide

nm	Nano meter
NSS	Normal saline solution
OECD	Organization for Economic Co-operation and Development
PCV	Packed cell volume
P.o	Post oral
RBC	Red blood cells
ROS	Reactive oxygen species
rpm	Rotation per minute
Sec	Second
SERM	Selective estrogen receptor modulator
SEM	Standard Error Mean
T.C	Total Count
TPB	Total Protein Bimding
TNF	Tumor necrosis factor
TLC	Total leukocyte count
WHO	World Health Organization

1.1 INTODUCTION

What is cancer

The body is made up of billions of tiny cells. Normally, cells grow and multiply in a tightly regulated fashion. New cells are only made when and where they are needed. When cancer occurs the cells' growth cycle goes haywire making them multiply uncontrollably. Cells become cancer cells because of damage to DNA. This leads to formation of a lump that may be benign or non-cancerous or aggressive in its growth.[1]

Cancer, also called malignancy, is an abnormal growth of cells[2]. It is one of the most worldwide spread diseases[3]. Cancer appears as a mass or ulcerates. Cancer is a **"great imitator"[4].** It represents a group of several diseases characterized by uncontrolled cell division and growth resulting in tumours, if malignant, may spread to other parts of the body. Neoplastic cells originate from differentiated and specialized cells through a process of regression and de differentiation to a simpler; more primitive stage which divides continuously unlike the normal parent cells. The characteristic properties of neoplasia include; sustained proliferation, evasion of growth suppressors, immortality, anaplasia , continued replication, angiogenesis, reprogramming energy metabolism, invasion, metastasis, and escaping immune destruction[5.]

The multistep process including initiation, promotion, and progression of carcinogenesis is a complicated process that results from excessive production of oxidative radicals, DNA alterations, and lastly the loss of the normal regulatory pathways between cell proliferation, differentiation, and apoptosis[6,7]. Progress made in cancer therapy has not been sufficient to minimize the annual death rates. The currently used conventional anticancer drugs are also toxic to normal cells in addition to being toxic to cancer cells[8]. Hence, there is a greater need for new effective and safe strategies in cancer control and therapy.

Prevention is the most practical strategy to control occurrence and spread of cancer. Cancer chemoprevention aims to stop or reverse the development and progression of precancerous cells through the use of non-cytotoxic nutrients and/or pharmacologically active agents. It is also important to provide various cancer chemopreventive natural agents with different specific molecular and cellular targets, which act through multiple mechanisms[9].

Metastasis:

Cancer can spread from its original site by local spread, lymphatic spread to regional lymph nodes or by haematogenous spread via the blood to distant sites, known as metastasis. When cancer spreads by a haematogenous route, it usually spreads all over the body. However, cancer 'seeds' grow in certain selected site only ('soil') as hypothesized in the *soil and seed hypothesis* of cancer metastasis[4].

1.2 Causes for Cancer:

Genetic Mutations (90-95%)

Inherited genetics (5-10%)

Chemicals- Tobacco, Alcohol, Smoking (25-30%)

Diet & exercise (30-35%)

Infection (15-20%)

Radiation (10%)

Heredity

Physical agents

Hormones

Autoimmune diseases

1.3 Cancer Pathophysiology

There are trillions of cells in the body. These cells have a tightly regulated cell cycle that controls their growth, maturity, division and death. During childhood normal cells divide faster to allow the person to grow. Once adulthood is

reached the cells divide to replace cells and to repair injuries. This cell division and growth is controlled by the cellular blue print or DNA and genes that lie within the cell's nucleus.

Cancer begins when cells in a part of the body start to grow out of control. All types of cancer, irrespective of their origin, occur due to this disturbed growth of cells that leads to formation of tumours and lesions. In addition, the cancer cells possess some rogue like properties:

- They have longer life spans and instead of dying continue to grow and form new, abnormal cells
- Cancer cells can also invade other tissues. This is something that normal cells cannot do. This property is called metastasis.
- Cancer cells grow into tumors that are supplied by a new network of blood vessels. This is called angiogenesis and is unique in maintaining the blood supply and supply of nutrients to the cancer cells[1].

1.4 Epidemiology of cancer:

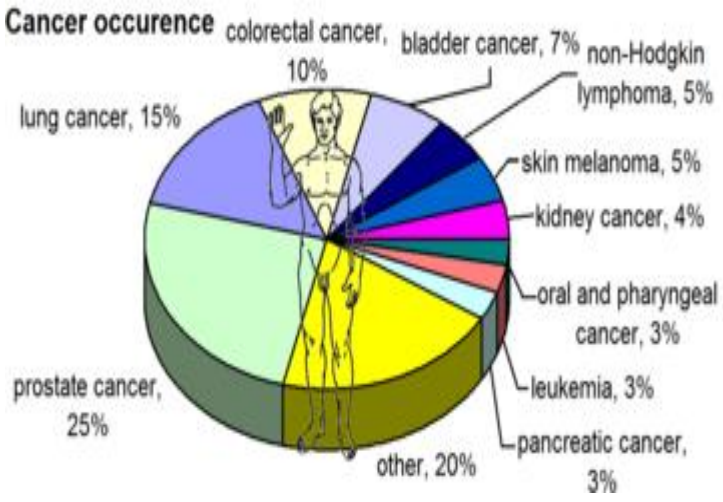
The epidemiology of cancer is the study of the factors affecting cancer, as a way to infer possible trends and causes. The study of cancer epidemiology uses epidemiological methods to find the cause of cancer and to identify and develop improved treatments[11].

Rates and mortality

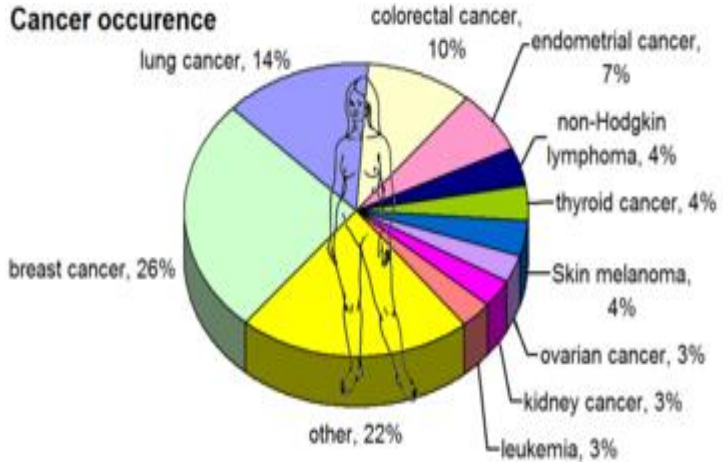
India:

According to the National Cancer Registry Programme of the India Council of Medical Research (ICMR), more than 1300 Indians die every day due to cancer. Between 2012 and 2014, the mortality rate due to cancer increased by approximately 6%. In 2012, there are 4,78,180 deaths out of 2934,314 cases reported. In 2013 there are 465,169 death out of 3016,628 cases. In 2014, 491,598 people died in 2014 out of 2820,179 cases[12]. As per Population Cancer Registry of Indian Council of Medical Research, the incidence and mortality of cancer is highest in the North Eastern region

of the country[13]. Breast cancer is the most common one, with Stomach cancer the leading cause of death by cancer for the population as a whole. Breast cancer and Lung cancer kill the most women and men respectively[14].



Most common cancers males, by occurrence[15]



In females, by occurrence[15]

Male		Female	
most common (by occurrence) [15]	**most common (by mortality) [15]**	**most common (by occurrence) [15]**	**most common (by mortality) [15]**
prostate cancer (25%)	lung cancer (31%)	breast cancer (26%)	lung cancer (26%)
lung cancer (15%)	prostate cancer (10%)	lung cancer (14%)	breast cancer (15%)
colorectal cancer (10%)	colorectal cancer (8%)	colorectal cancer (10%)	colorectal cancer (9%)
bladder cancer (7%)	pancreatic cancer (6%)	endometrial cancer (7%)	pancreatic cancer (6%)
non-Hodgkin lymphoma(5%)	liver & intrahepatic bile duct (4%)	non-Hodgkin lymphoma(4%)	ovarian cancer (6%)
skin melanoma (5%)	leukemia (4%)	thyroid cancer (4%)	non-Hodgkin lymphoma (3%)
kidney cancer (4%)	esophageal cancer (4%)	Skin melanoma (4%)	leukemia (3%)
oral and pharyngeal cancer(3%)	bladder cancer (3%)	ovarian cancer (3%)	uterine cancer (3%)
leukemia (3%)	non-Hodgkin	kidney cancer (3%)	liver & intrahepatic bile

	lymphoma(3%)		duct (2%)
pancreatic cancer (3%)	kidney cancer (3%)	leukemia (3%)	brain and other nervous system (2%)
other (20%)	other (24%)	other (22%)	other (25%)

Children:

Childhood cancer and cancer in adolescents is rare (about 150 cases per million yearly in the US). Leukemia (usually acute lymphoblastic leukemia) is the most common cancer in children aged 1–14 in the U.S., followed by the central nervous system cancers, neuroblastoma, Wilms' tumor, and non-Hodgkin's lymphoma[15].
Statistics from the SEER program of the US NCI demonstrate that childhood cancers increased 19% between 1975 and 1990, mainly due to an increased incidence in acute leukemia. Since 1990, incidence rates have decreased[16.]

Infants:

Neuroblastoma comprised 28% of infant cancer cases and was the most common malignancy among these young children (65 per million infants)[16].
The leukemias as a group (41 per million infants) represented the next most common type of cancer, comprising 17% of all cases[16].
Central nervous system malignancies comprised 13% of infant cancer, with an average annual incidence rate of nearly 30 per million infants[16].
The average annual incidence rates for malignant germ cell and malignant soft tissue tumors were essentially the same at 15 per million infants. Each comprised about 6% of infant cancer[16].

What makes a normal cell turn cancerous:

A normal cell can become a cancer cell if it undergoes damage to the DNA. Since it is the DNA that regulates the cells' cycle of growth and death and any changes or damage to DNA affects the cell.

For most cells if the DNA is damaged the cell either repairs the damage or the cell dies. In cancer cells, the damaged DNA is not repaired and the damage is propagated to newer abnormal cells that are born of the defective cell.

Damaged DNA by mutation can also be inherited from parents or relatives.

DNA damage can also occur due to exposure to toxins like cigarette smoking, alcohol etc.

Normal cell transform into a cancer cells when Gene regulate cell growth and differentiation is altered[1].

The affected genes are divided into two broad categories.

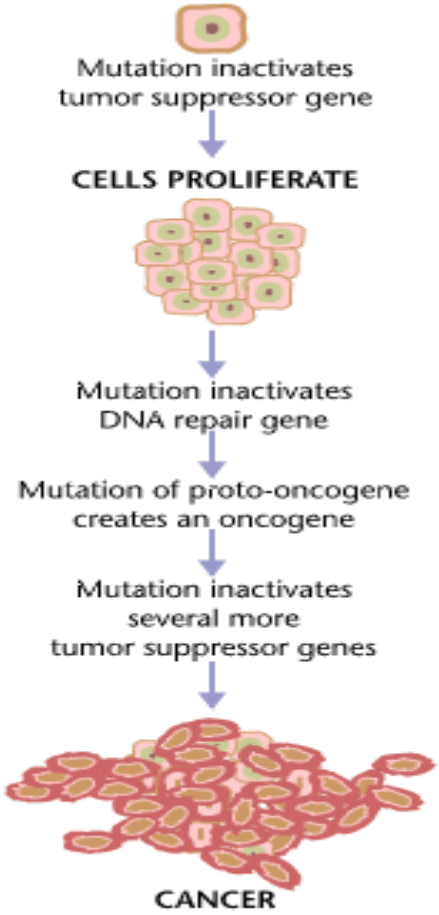
- Oncogenes

  Oncogenes are genes that promote cell growth and reproduction.

- Tumor suppressor gene

  Tumor suppressor genes are genes that inhibit cell division and survival[17].

Genetic changes can occur at different levels and by different mechanisms. The gain or loss of an entire chromosome can occur through errors in mitosis. More common are mutations, which are changes in the nucleotide sequence of genomic DNA[18].



1.5 Types of cancers[19]:

There are more than 200 types of cancers.

But most common types of cancers are as follows:

Breast cancer:

A cancer that forms in the cells of the breasts.

Prostate cancer:

A cancer in a man's prostate, a small walnut-sized gland that produces seminal fluid.

Basal cell cancer:

A type of skin cancer that begins in the basal cells.

Melanoma:

The most serious type of skin cancer.

Colon cancer:

A cancer of the colon or rectum, located at the digestive tract's lower end.

Lung cancer:

A cancer that begins in the lungs and most often occurs in people who smoke.

Leukemia:

A cancer of blood-forming tissues, hindering the body's ability to fight infection.

Lymphoma:

A cancer of the lymphatic system.

Oral Cancer:

Cancer that develops in any part of the mouth

Cervix Cancer:

Cancer that develops in the cervix part.

1.6 Signs & Symptoms:

Symptoms vary widely or they may not occur at all. Some patients have abnormal bumps, unexplained fevers, night sweats or unintentional weight loss, fatigue, pain, skin changes, change in bowel or bladder function, unusual bleeding, persistent cough or voice change, lumps, or tissue masses, hair loss[20, 21].

1.7 Management:

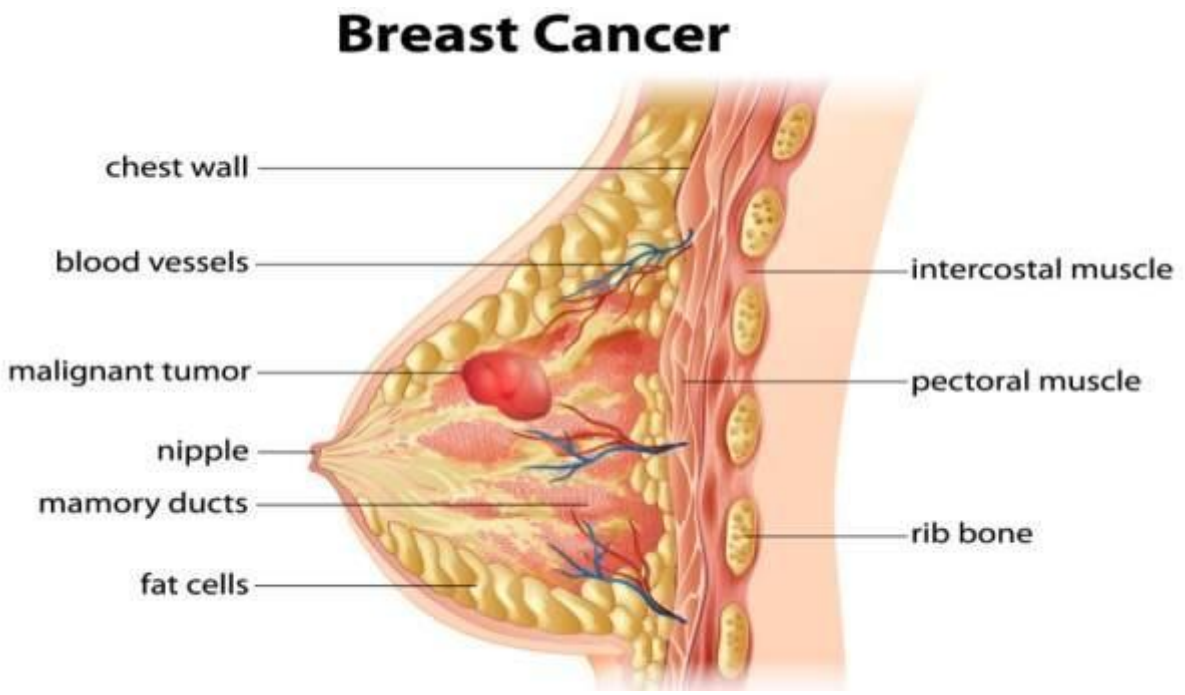
Line of treatment of cancer includes chemotherapy, radiotherapy as well as surgery. Patient survival rate depends upon the type, location and stage of the disease condition at the onset of treatment. Chemotherapy along with surgery has been quite successful in a number of different types of cancer including breast, pancreatic, colorectal, osteogenic sarcoma, ovarian, testicular as well as certain lung cancers. However, effectiveness of chemotherapy is often limited by the toxicity on other non-target tissues. Therefore, complementary and alternative safer therapies such as herbal therapies are becoming increasingly more popular[22].

2.1 What is breast cancer

Breast cancer is a malignant tumor that starts in the cells of the breast. A malignant tumor is capable of invading surrounding tissues or spreading or metastasizing to distant areas of the body. The disease occurs almost entirely in women, but men can get it, too[23, 24].

Breast cancer is the most common malignant disease and the leading cause of cancer-related death in women around the world. Worldwide, it is estimated that >1 million women are diagnosed with breast cancer every year, and that >40% of these patients die from the disease, representing 14% of female cancer deaths[23, 25].

In the United States, breast cancer is the second leading cause of cancer death among women. The American Cancer Society has estimated that 89% of women survive 5 years after invasive breast cancer diagnosis[5, 4].



Endocrine therapy targets estrogen receptor-positive breast cancers and represents the most effective treatment for them. In the last four decades, several hormonal agents have been used in palliative settings for advanced cancer and as adjuvant therapy to prevent the recurrence. However, there are several proposed molecular mechanisms that explain endocrine resistance in a large proportion of patients, which limit the effectiveness of endocrine treatments[28, 29,30] .

Breast cancer patients with the same stage of disease can have markedly different responses and overall outcome. The strongest predictors for metastases (for example, lymph node status and histological grade) fail to classify accurately breast tumours according to their clinical behaviour[31]. Chemotherapy or hormonal therapy reduces the risk of distant metastases by approximately one-third; however, 70–80% of patients receiving this treatment would have survived without it. None of the signatures of breast cancer gene expression reported to date, allow for patient-tailored therapy strategies[32].

Mutations in the p53 gene are associated with a wide variety of human tumors, including those of the breast. To assess functionally the role of the p53 gene in the development of human breast cancer[33].

Breast cancer occurs when a malignant (cancerous) tumor originates in the breast. As breast cancer tumors mature, they may metastasize (spread) to other parts of the body. The primary route of metastasis is the lymphatic system which, ironically enough, is also the body's primary system for producing and transporting white blood cells and other cancer-fighting immune system cells throughout the body. Metastasized cancer cells that aren't destroyed by the lymphatic system's white blood cells move through the lymphatic vessels and settle in remote body locations, forming new tumors and perpetuating the disease process.

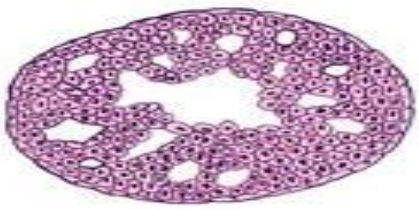
Breast cancer is fairly common. Because of its well-publicized nature, and potential for lethality, breast cancer is arguably the most frightening type of cancer diagnosis someone can receive. However, it is important to keep in mind that, if identified and properly treated while still in its early stages, breast cancer can be cured.

Breast cancer is not just a woman's disease. It is quite possible for men to get breast cancer, although it occurs less frequently in men than in women[34].

2.2 Types of breast cancer:

There are several varieties of breast cancer. These can affect various parts of the breast. Breast cancer is often divided into non-invasive and invasive types.

Non-invasive breast cancer:



Non-invasive breast cancer is also known as cancer or carcinoma in situ, or pre-cancerous cells. This is seen in the ducts of the breast and does not have the ability to spread outside the breast. This form of cancer rarely shows as a lump in the breast and is usually found on a routine check up with a mammogram. The most common type of non-invasive cancer is ductal carcinoma in situ (DCIS)[35, 36]. DICS is classified as Stage 0[37]. It is the most common type of pre-cancer in women[38].

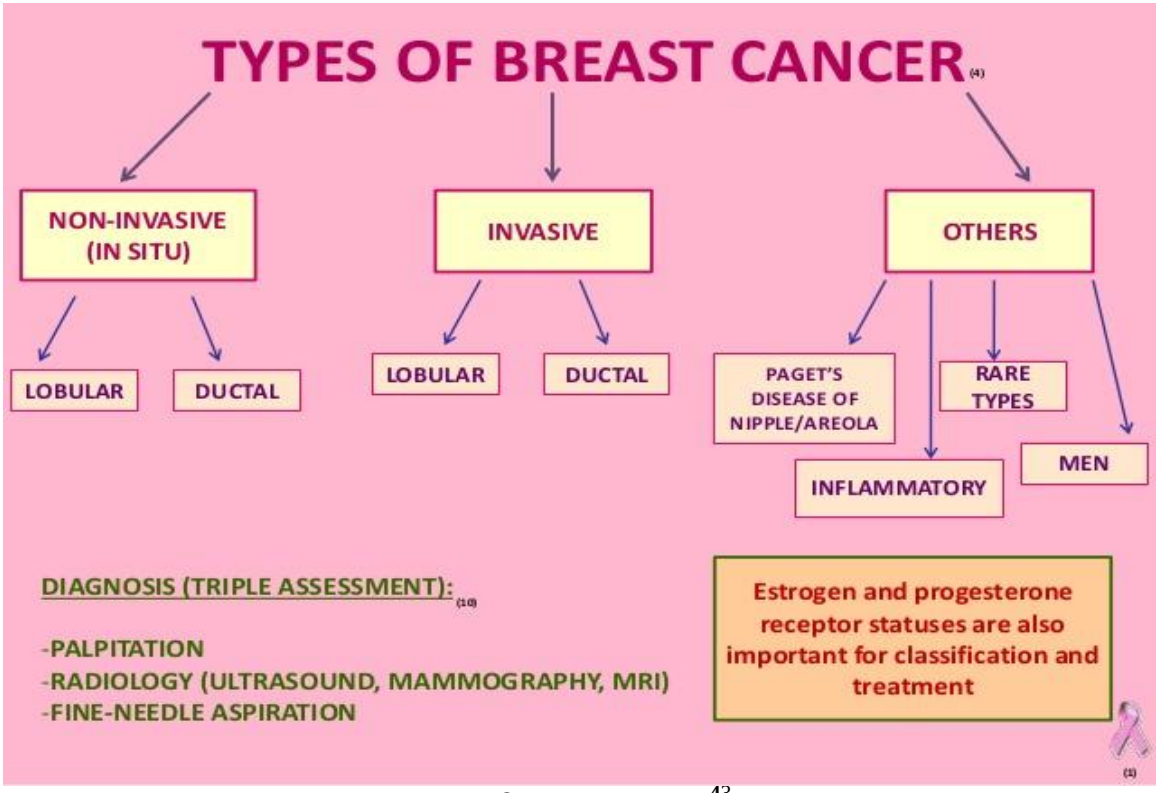
Invasive cancer:

Invasive cancer is more aggressive and spreads outside the breast. The most common form of breast cancer is invasive ductal breast cancer. This type

develops around the ducts of the breast and accounts for about 80% of all cases of breast cancer and is sometimes called 'no special type'[35].

Other types of breast cancer

Less common varieties of breast cancer include invasive lobular breast cancer (5-10%),[39,40] inflammatory breast cancer[41] and Paget's disease of the breast[42].



Types of Breast cancer [43]

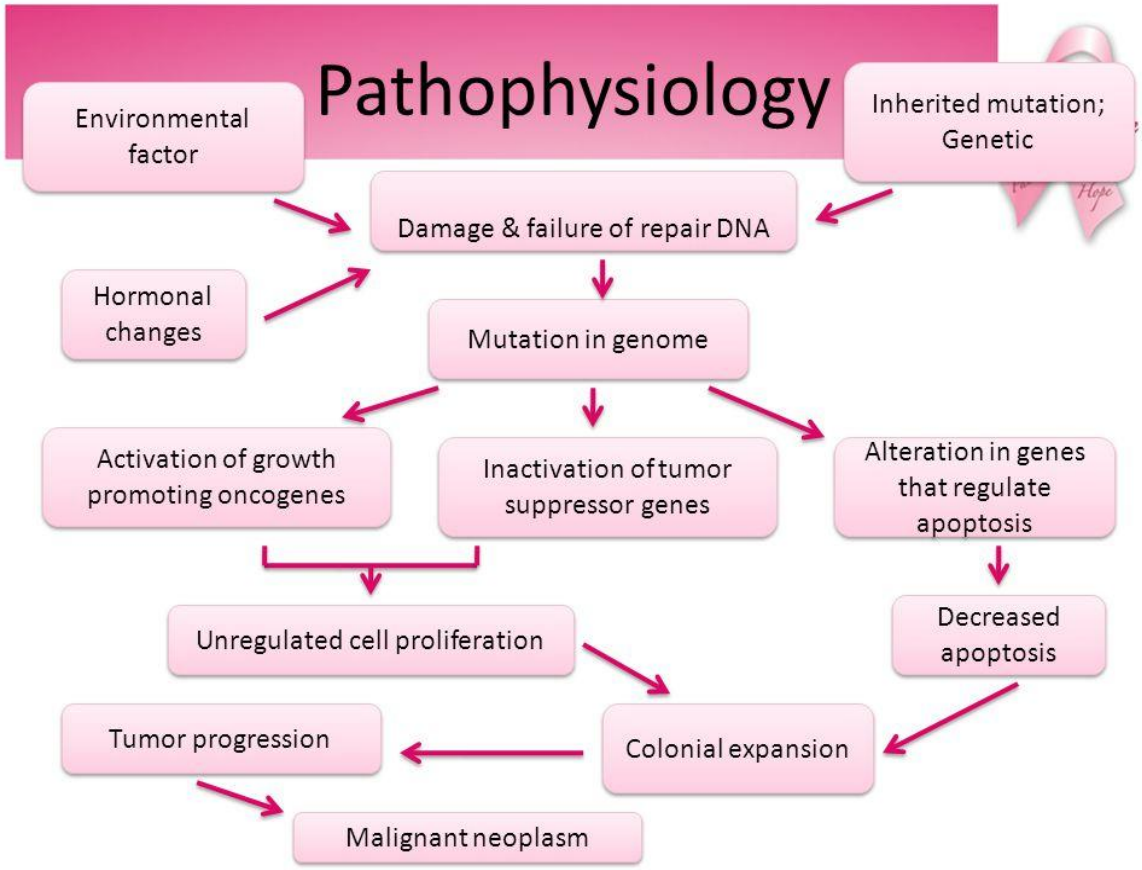
2.3 Breast Cancer Pathophysiology:

Breast cancer is a malignant tumor that starts in the cells of the breast. Like other cancers, there are several factors that can raise the risk of getting breast cancer. Damage to the DNA and genetic mutations can lead to breast cancer have been experimentally linked to estrogen exposure. Some individuals inherit

defects in the DNA and genes like the BRCA1, BRCA2 and P53 among others. Those with a family history of ovarian or breast cancer thus are at an increased risk of breast cancer[44, 45, 46].

The immune system normally seeks out cancer cells and cells with damaged DNA and destroys them. Breast cancer may be a result of failure of such an effective immune defence and surveillance.

These are several signalling systems of growth factors and other mediators that interact between stromal cells and epithelial cells. Disrupting these may lead to breast cancer as well[47, 48].



Breast Cancer Pathophysiology[49]

2.4 Breast Cancer Epidemiology:

Breast cancer is the most common cancer in women worldwide after skin cancer[50]. It represents 16% of all cancers in women. This rate is twice that of colorectal cancer and cervical cancer and about three times that of lung cancer. Death rates are also 25% greater than that of lung cancer in women[51, 52].

Incidence of breast cancer in India:

Over 100,000 breast cancer patients are estimated to be diagnosed annually in India[53].

Incidence of breast cancer around the world

All around the world the incidence of this cancer shows varied rates. The rates are low in less-developed countries and greatest in the more-developed countries. Breast cancer is related to age with only 5% of all breast cancers occur in women under 40 years old[54].

In the twelve world regions, the annual age-standardized incidence rates per 100,000 women are as follows[53]:

- Eastern Asia – 18 per 100,000 women
- South Central Asia - 22 per 100,000 women
- Sub-Saharan Africa – 22 per 100,000 women
- South-Eastern Asia – 26 per 100,000 women
- North Africa - 28 per 100,000 women
- Western Asia - 28 per 100,000 women
- South and Central America - 42 per 100,000 women
- Eastern Europe - 49 per 100,000 women
- Southern Europe - 56 per 100,000 women

- Northern Europe - 73 per 100,000 women
- Oceania - 74 per 100,000 women
- Western Europe - 78 per 100,000 women
- North America - 90 per 100,000 women

2.5 Epidemiology of Breast Cancer:

Worldwide, breast cancer is the most common invasive cancer in women. (The most common form of cancer is non-invasive non-melanoma skin cancer; non-invasive cancers are generally easily cured, cause very few deaths, and are routinely excluded from cancer statistics.) Breast cancer comprises 22.9% of invasive cancers in women and 16% of all female cancers[55, 56].

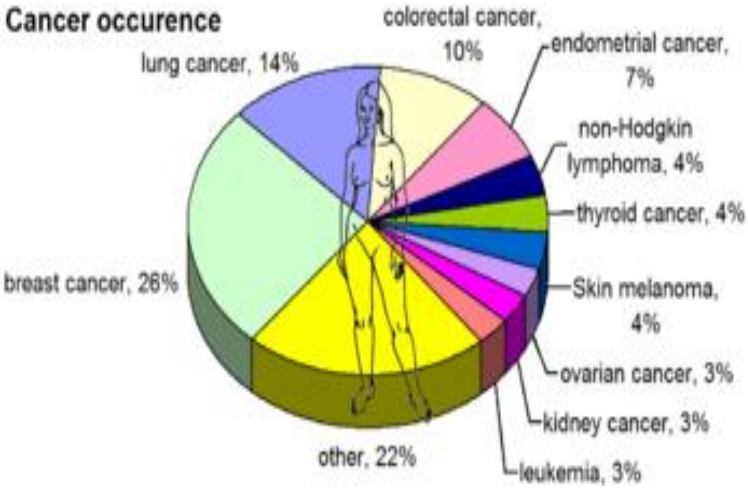
In 2008, breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women and 6.0% of all cancer deaths for men and women together). Lung cancer, the second most common cause of cancer-related death in women, caused 12.8% of cancer deaths in women (18.2% of all cancer deaths for men and women together)[55]

By age group:

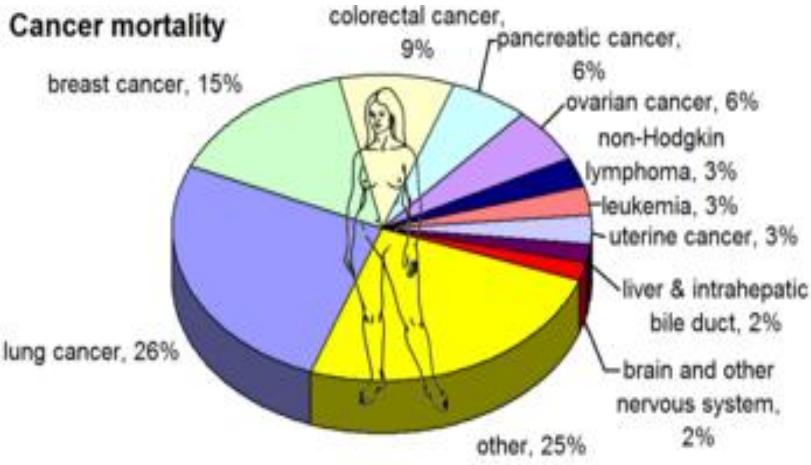
Breast cancer is strongly related to age, with only 5% of all breast cancers occurring in women under 40 years old[57].

By region:

The incidence of breast cancer varies greatly around the world: it is lowest in less-developed countries and greatest in the more-developed countries. In the twelve world regions, the annual age-standardized incidence rates per 100,000 women are as follows: in Eastern Asia, 18; South Central Asia, 22; sub-Saharan Africa, 22; South-Eastern Asia, 26; North Africa and Western Asia, 28; South and Central America, 42; Eastern Europe, 49; Southern Europe, 56; Northern Europe, 73; Oceania, 74; Western Europe, 78; and in North America, 90[58]



Cancer occurrence in females[59]



Cancer occurrence in women[59]

2.5 Causes for Breast Cancer:

The causes of breast cancer are not yet definitively known. However, extensive research efforts have uncovered various risk factors that are associated with increased incidence of breast cancer in women. Though some of these risk factors are unavoidable and uncontrollable, some of them are very avoidable, making it possible for people to take action so as to minimize their cancer risk. Even a minimized risk is still a risk, however. There is no way to pre-determine whether a person will get breast cancer until they have either been diagnosed with it or they have lived a breast-cancer free lifetime[60,61].

Some of the risk factors for breast cancer that are difficult or impossible to control include:

- **Age:** As women age their chance of getting breast cancer increase. The majority of breast cancer cases are diagnosed in women over the age of 50[62].

- **Family and genetic history**: Some cases of breast cancer are known to have been caused as a result of genetic mutation. Gene mutations can be inherited from parents and can also occur in otherwise healthy people in response to environmental toxins. Having a close relative on either side of your family who has breast cancer will, in general, increase your risk of contracting breast cancer. This is particularly true if the relative is what is called "first degree" meaning your sister or mother. In general, the more first degree relatives that have had breast cancer the more likely you are to develop breast cancer as well[63].

- **BRCA gene mutation**. Recent advances in genetic mapping have discovered a gene mutation that increases the risk of certain female cancers in women with the mutation. The two main breast and ovarian cancer susceptibility genes are known as BRCA1 and BRCA2[64].

• **Previous breast cancer.** A woman who previously had breast cancer and has been cured has a greater chance for having a new breast cancer episode occur than is a woman who never had the disease. New tumors appearing inside someone with a previous history of cancer who has been cancer free are not necessarily related to or caused by old eradicated tumors. Instead, new tumors can form spontaneously in vulnerable persons[65].

• **Race.** Hispanic and Asian women have lower risk for getting breast cancer than do Caucasian and African American women. In fact, Caucasian women are at the highest risk of getting the disease compared to women of other racial backgrounds. . It may occur because, as a group, these women have less access to healthcare, or possibly because they are less likely as a group to seek healthcare. Research is ongoing in this area to determine the cause of this alarming disparity[62].

• **Previous benign tumor biopsy results.** Women who have had previous biopsy that has found a benign (non-cancerous) tumor in the breast are at a greater risk for malignant breast tumors in the future. However, the occurrence of cysts in the breast does not increase the risk for future breast cancer[65].

• **Prior radiation treatment in the chest area.** Women who receive prior radiation treatment directed at their chest area for any reason are at a heightened risk for future breast cancer compared to women who do not receive radiation treatment. The earlier in life women are exposed to chest-targeted radiation treatment, the higher their later risk for breast cancer. Radiation exposure from various sources including medical treatment and nuclear explosion increases the risk of breast cancer[65].

• **Menstrual Cycles.** Women who begin menopause after the age of 50 or who had their first menstrual cycle before age 12 run a slightly higher risk for breast cancer than women whose menstrual cycles started after age 12 and end prior to age 50. This is due to prolonged exposure to high levels of certain reproductive hormones[66].

While the above risk factors for breast cancer are difficult or impossible to avoid, there are also numerous risk factors for breast cancer that can be avoided by making healthy lifestyle choices. While choosing a healthy lifestyle may not always be simple or easy, making such lifestyle changes can help you lower your cancer risk with regard to the following risk factors

• **Use of Birth Control Pills.** Some studies have shown that women who use birth control pills are at a slightly increased risk for breast cancer. However, any relationship between breast cancer and birth control pill use remains controversial at this time, pending further research. It's not all bad news. While oral contraceptives (birth control pills) may slightly increase risk of breast cancer[67].

• **Not Having Children.** Women who have their first child after the age of 30 or who have never had children run a slightly higher risk for contracting breast cancer than do women who give birth before reaching age 30. This is again due to more prolonged exposure to reproductive hormones[68].

• **Hormone Replacement Therapy.** Hormone replacement therapy (HRT) is sometimes prescribed to women as a means of alleviating discomfort associated with menopause. Research indicates that women who have received HRT for five years or more may be at a heightened risk for breast cancer. However, the heightened risk seems to occur primarily in women using combined (estrogen and progesterone mixture) HRT, as opposed to estrogen-only HRT. However, estrogen-only HRT increases risk of uterine cancer. The elevated breast cancer

risk appears to be reversible in that women who discontinued HRT for five or more years show no more increased risk for breast cancer than women who never used it in the first place. There are real benefits to HRT that should not be dismissed lightly just because of the elevated breast cancer risk it may come with. Talk to your doctor about your risks and benefits with regard to HRT. HRT may be a good idea for you if you are at a low risk for breast cancer and could benefit from its therapeutic effects. However, if you are already at a high risk for breast cancer you should carefully weigh the risks and benefits before beginning HRT[69,70].

- **Failing To Exercise.** Some recent studies have found a small relationship between moderate exercise and decreased risk of breast cancer. Exercising regularly can help combat obesity as well which further lowers cancer risk[71].

- **Breast Feeding.** Multiple studies have demonstrated that breast feeding is associated with reduced risk of breast cancer. In addition, the longer you breast feed the better the cancer protection you appear to achieve[72].

- **Excessive Alcohol Intake.** Drinking alcohol has been shown to slightly increase the risk of breast cancer[65].

- **Obesity and Poor Diet.** Research has established a link between being overweight and an elevated risk of postmenopausal breast cancer. This seems to be because the majority of reproductive hormones in postmenopausal women is produced in the fat cells. Therefore, the more fat cells a woman has the higher their reproductive hormone levels[73].

- **Exposure to Diethylstilbesterol (DES):** Women who took diethylstilbestrol (DES) to prevent miscarriage may have an increased of breast cancer after age 40[74].

2.6 Sign & Symptoms of Breast Cancer[75, 76, 77] :

Breast cancer is a disease that progresses in stages, building in intensity over time. The early stages of breast cancer there may be no discernable symptoms. Early breast cancers may be asymptomatic, and pain and discomfort are typically not present. If a lump is discovered, the following may indicate the possible presence of breast cancer.

During the early and middle stages of breast cancer symptoms can include the following:

- A lump, dimple or thickening in the breast
- A lump or swelling in the armpit (an enlarged lymph node)
- Change in the shape of the breast
- Change in the size of the breast, including swelling
- Change in the color or texture of the breast and/or nipple, including redness, scaly, dimpled, retracted, or puckered appearance
- Breast pain, especially if in one breast only
- Abnormal discharge from the nipple.

Diagnosis:

Breast cancer is often first detected as an abnormality on a mammogram before it is felt by the patient or health care provider.

Evaluation of breast cancer includes the following:

- Clinical examination
- Imaging
- Needle biopsy

Physical examination

The following physical findings should raise concern:

- Lump or contour change
- Skin tethering
- Nipple inversion
- Dilated veins
- Ulceration
- Paget disease
- Edema or peau d'orange

If a palpable lump is found and possesses any of the following features, breast cancer may be present:

- Hardness
- Irregularity
- Focal nodularity
- Fixation to skin or muscle

Screening

Early detection remains the primary defense in preventing breast cancer. Screening modalities include the following:

- Breast self-examination
- Clinical breast examination
- Mammography
- Ultrasonography
- Magnetic resonance imaging

Ultrasonography and MRI are more sensitive than mammography for invasive cancer in nonfatty breasts. Combined mammography, clinical examination, and MRI are more sensitive than any other individual test or combination of tests.

Biopsy

Core biopsy with image guidance is the recommended diagnostic approach for newly diagnosed breast cancers. This is a method for obtaining breast tissue without surgery and can eliminate the need for additional surgeries. Open excisional biopsy is the surgical removal of the entire lump.

2.7 Screening of Breast cancer:

There are several methods to screen breast cancer.

• **Clinical Breast Exam.** During a clinical breast exam (CBE for short) a doctor first observe your breasts for any inequalities or changes in size or shape, then palpates (feels) your breasts and armpits looking for lumps and swellings[78].

• **Mammogram.** A mammogram is basically an x-ray image of the breast's interior tissues and any lumps or irregularities that may exist therein. During this procedure breast is placed between two plates which are then slightly compressed. Your breast is then subject to a beam of radiation and an x-ray picture is taken[79].

• **Axillary Dissection and Biopsy.** An axillary dissection involves a small surgery wherein cells from the lymph nodes in the armpit are removed and then studied under the microscope to determine if they are cancerous. The general name for this tissue sampling and study is "biopsy"[79].

• **Hormone receptor testing**. Recent advances in testing of biopsy specimens allow doctors to test tumor samples for certain hormone receptors. If these hormone receptors are present in the tumor it allows doctors to use certain medications to help fight the cancer and can improve a person's likelihood of survival[80].

2.8 Treatment for Breast Cancer:

There are two general approaches to treating cancers: **local treatment and systemic (whole body) treatment.**

Local treatment involves treating just the small areas of the body containing tumors while affecting the rest of the body as little as possible.

Systemic treatment approaches, on the other hand, are used to treat cancer that has spread throughout the body.

Local treatment approaches alone may be all that is required for treatment of early stage breast cancer (e.g., before metastasis - the spreading of cancerous tissues through the body -- has occurred).

Combined local and systemic approaches may be necessary for treatment of more advanced cancers. Surgical approaches tend to fall into the local treatment category, while chemotherapy approaches fall into the systemic category.

Radiation therapy approaches can be local or systemic depending on how they are administered.

The majority of women who are afflicted by breast cancer will require some type of surgery. The goal of the surgical procedure is to remove the cancerous tumor while preserving as much of the healthy breast tissue as possible. Later stage breast cancers often require more extensive surgeries as more tissues get involved in the disease process[81].

Some of the surgical procedures used to treat breast cancer are:

• **Lumpectomy.** Lumpectomy is a surgical procedure in which the breast tumor is removed while sparing as much healthy surrounding tissue as possible. The benefit of lumpectomy is that in most circumstances the mass and form of the breast can be retained. Radiation treatment and chemotherapy are often given in conjunction with lumpectomy. Recovery time from a lumpectomy is very short[82].

• **Partial Mastectomy.** This surgical procedure is similar in principle to a lumpectomy but involves removing more of the tissue surrounding the cancerous tumor. Partial mastectomy can also be followed by radiation therapy or chemotherapy[83].

• **Modified Radical Mastectomy.** This surgical procedure involves removing the entire breast mass and involved lymph nodes from under the arm (while preserving as many of the lymph nodes as possible). Understandably, removal of the entire breast and the disfigurement this creates can be traumatic and depressing for many women. Reconstructive surgery may be desirable[83].

• **Radical Mastectomy.** During this surgical procedure the entire breast is removed as well as the lymph nodes under the arm and the pectoral (chest) muscles under the breast[84].

Surgical removal of the tumor causing a cancer is often not sufficient for a full recovery, particularly when metastasis has occurred. It is common to see systemic chemotherapy or radiation therapies applied in conjunction with surgery. Chemotherapy and radiation therapies offer doctors a way to destroy any cancer cells in the surrounding area of the main tumor that they might have missed during surgery.

- **Hormonal therapy.** Tumor biopsy specimens can be tested for specific hormone receptors it has allowed scientists to develop drugs which can block those receptors and slow a tumor's growth. The main type of drug used blocks the effects of a hormone called estrogen[80].

2.9 Management:

Surgery and radiation therapy, along with adjuvant hormone or chemotherapy when indicated, are now considered primary treatment for breast cancer. Surgical therapy may consist of lumpectomy or total mastectomy. Radiation therapy may follow surgery in an effort to eradicate residual disease while reducing recurrence rates. There are 2 general approaches for delivering radiation therapy:

- External-beam radiotherapy (EBRT)
- Partial-breast irradiation (PBI)

Surgical resection with or without radiation is the standard treatment for ductal carcinoma in situ[85, 86].

Pharmacologic agents

Hormone therapy and chemotherapy are the 2 main interventions for treating metastatic breast cancer. Common chemotherapeutic regimens include the following:

- Docetaxel
- Cyclophosphamide
- Doxorubicin
- Carboplatin
- Methotrexate
- Trastuzumab

Two selective estrogen receptor modulators (SERMs), tamoxifen and raloxifene, are approved for reduction of breast cancer risk in high-risk women. HER2-targeted therapies have been investigated in combination with angiogenesis inhibitors, with promising results. HER2 overexpression is

associated with an increase in VEGF levels in primary breast cancers. Dual blockade by antiangiogenic/HER2 agents (eg, neratinib) targeting HER2, and EGFR pathways produces greater inhibition of human breast cancer cell lines. In patients receiving adjuvant aromatase inhibitor therapy for breast cancer who are at high risk for fracture, the monoclonal antibody denosumab or either of the bisphosphonates zoledronic acid and pamidronate may be added to the treatment regimen to increase bone mass. These agents are given along with calcium and vitamin D supplementation[87].

2.10 Herbs as alternatives to anticancer therapy[88]:

The use of herbs as anticancer botanicals has been in practice since ancient times that began with folk medicine and by the time has been adopted in traditional and allopathic medicine. Many drugs currently used in chemotherapy were either isolated from plant species or derived from their natural precursors. Vincristine and vinblastine (*Vinca* alkaloids) isolated from *Catharanthus roseus*; semisynthetic derivatives of podophyllotoxin *viz.* etoposide and teniposide, derived from *Podophyllum* spp.; taxanes isolated from *Taxus* spp., camptothecin, irinotecan and topotecan, from *Camptotheca acuminata*, and many others are few examples. According to a study, over 50 % of the anticancer drugs in clinical trials are directly or indirectly derived from plants.

Plant-derived drugs

Anticancer agent	**Isolated or derived from:**	**Compound activity**
Sulphoraphane	Isotiocynanate in cruciferous vegetables *Brassica*	Induces phase 2 detoxification enzymes; inhibits tumor growth in breast cancers; antiproliferate effects
Paclitaxel (Taxol)	Taxane; *Taxus brevifolia*L	Microtubule disruptor; block mitosis; induce apoptosis; microtubules are polymerized and stabilized; disruption of spindle formation; inhibition of translational machinery
Epipodophyllotoxin	*Podophyllum peltatum*L.; Podophyllotoxin isomer	Pro-apoptotic effects; cell cycle interference
Vincristine	*Catharanthus roseus* G.	Anti-mitotic; microtubule

Vinblastine	Don; Vinca alkaloids	inhibitor; bind to β-tubulin; microtubule stabilizers or destabilizers; pro-apoptotic properties and induce cell cycle arrest; anti-tumour activity
Vinorelbine		
Vindesine		
Vinflunine		
Pomiferin	Isoflavonoid isolatated from *Maclura pomifera; Dereeis Malaccensis*	Pro-apoptotic effects; DNA fragmentation; inhibits oxidative damage of DNA; antioxidant activity; inhibits histone deacetylases; cytotoxicity of cancer cells
Epigallacotechin -3-gallate	Catechin; green tea	Antioxidant; decrease DNA damage from oxidative stress; anti-proliferative effects; inhibition of specific kinases; inhibit carcinogenesis induced chemically or by UV

Combretastatin A-4 phosphate	Water-soluble analogue of combretastatin; *Combretum caffrum*	Anti-angiogenic; vasuclar shut-down of tumors; tumor necrosis
Roscovitine	Derived from olomucine; *Raphanus sativus* L. (*Brassicaceae*)	Inhibition of cyclin dependent kinases; reduction of cell cycle progression
Flavopiridol	Synthetic flavonoid derivative; rohitukine based structure; *Dysoxylum binectariferum*Hook.f. (*Meliaceae*)	Anti-inflammatory; immunamodulatory activity; tyrosine kinase activyt; growth inhibitory effects
Noscapine	Opium poppy (*Papaver somniferum*)	Antiproliferative properties; microtubule interfering; inhibits tumour growth and progression

Antioxidants are chemical substances derived from natural sources or from synthetic origin, protect the body from oxidative stress either by preventing the formation of ROS or by interfering ROS attack by scavenging the reactive intermediates or by converting to less reactive molecules. The antioxidant capacity tells about the duration whereas the activity denotes the starting

dynamics of antioxidative mechanism. Therefore, uses of antioxidants, both natural and synthetic are gaining widespread importance in prevention of diseases[89].

Many natural products obtained from medicinal plants, or secondary metabolites such as alkaloids, terpenoids, phenolic acids, flavonoids, tannins, lignins, quinones, coumarins which exhibit potential antioxidant and other properties, have played an important role in treatment of cancer. Studies suggest that antioxidants possess anti-inflammatory, antimutagenic and anti-carcinogenic activities. Medicinal plants thus can be very promising in improving the present and future health needs against cancer. This is because of their secondary metabolites which can maintain the health and cure of various diseases including cancer with less harmful effects)[89].

Eighty percent of world's population uses plants to cure disease, so the WHO (World Health Organization) recommends the use of medicinal plants in primary health care systems but based on scientific evidence to assure the safety, effectiveness, and quality required[89].

What are free radicals, and do they play a role in cancer development :[88,89]

Free radicals are highly reactive chemicals that have the potential to harm cells. They are created when an atom or a molecule (a chemical that has two or more atoms) either gains or loses an electron (a small negatively charged particle found in atoms). Free radicals are formed naturally in the body and play an important role in many normal cellular processes. At high concentrations, however, free radicals can be hazardous to the body and damage all major components of cells, including DNA, proteins, and cell membranes. The damage to cells caused by free radicals, especially the damage to DNA, may play a role in the development of cancer and other health conditions.

Abnormally high concentrations of free radicals in the body can be caused by exposure to ionizing radiation and other environmental toxins. When ionizing radiation hits an atom or a molecule in a cell, an electron may be lost, leading to the formation of a free radical. The production of abnormally high levels of free radicals is the mechanism by which ionizing radiation kills cells. Moreover, some environmental toxins, such as cigarette smoke, some metals, and high-oxygen atmospheres, may contain large amounts of free radicals or stimulate the body's cells to produce more free radicals.

Free radicals that contain the element oxygen are the most common type of free radicals produced in living tissue. Another name for them is "reactive oxygen species," or "ROS"

What are antioxidants?

Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing damage. Antioxidants are also known as "free radical scavengers."

The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants.

However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants. Some dietary antioxidants are also available as dietary supplements.

Examples of dietary antioxidants include beta-carotene, lycopene, and vitamins A, C, and E (alpha-tocopherol). The mineral element selenium is often thought to be a dietary antioxidant, but the antioxidant effects of selenium are most likely due to the antioxidant activity of proteins that have this element as an essential component (i.e., selenium-containing proteins), and not to selenium itself .

Can antioxidant supplements help prevent cancer?

In laboratory and animal studies, the presence of increased levels of exogenous antioxidants has been shown to prevent the types of free radical damage that have been associated with cancer development.

Antioxidant supplementation and cancer prevention:

Experimental Intervention	Sample Size	Duration Of Study	Characteristics Of Participants	Study Outcome
B-carotene: 15mg/day Vit E: 30mg/day Selenium: 50mcg/day	29,584	6 years	Malnourished Age: 40 - 69	Lower cancer rate
B-carotene: 20mg/day compared to atocopherol: 50mg/	29,133	5 - 8 years Average 6 years	Male smokers Age: 50 - 69	B-carotene: 20mg/day associated with increase in lung

day				cancer
B-carotene: 30mg/day and retinyl palmitate: 25 000IU/day	18,314	4 years	Male and female Age: 45 - 74 Smoker and/or occupational exposure to asbestos	No clear evidence of benefit. Associated with more death
B-carotene: 50mg on alternate days	22,071	11.6 - 14.2 years, average 12 years	Male Age: 40 - 84 Current/former or non-smokers	No clear evidence of benefit or harm related to cancer risk
B-carotene: 50mg on alternate days	39,876	0.00-2.72 years, median 2.1 years	Female Age: > = 45	No significance difference in incidence of cancer
B-carotene: 25mg/day Vit C: 1000mg/day Vit E: 400IU/day	864	4 years	Adenoma diagnosed Good health Age: < 80	No clear evidence of benefit
B-carotene: 50mg/day	1,805	5 years	Recent non-melanoma skin cancer	No clear evidence of benefit

Piper aduncum Capsule on DMBA-induced Breast Cancer in Rats

Arroyo-Acevedo J et al, investigated that the protective effect of *Piper aduncum* capsule on DMBA (dimethylbenz[α]anthracene)-induced breast cancer in rats. It was assessed by monitoring the tumor and lung metastases incidence and recording hematological and biochemical parameters. There was a significant decrease in the frequency of DMBA-induced micronucleated polychromatic erythrocyte ($P < 0.01$). Considering the antitumorigenic, hypolipidemic, anti-inflammatory, antioxidant, and antigenotoxic properties of *P. aduncum* capsule.

Breast Cancer (Auckl). 2015 Jun 29;9:41-8. doi: 10.4137/BCBCR.S24420.

Ulva lactuca polysaccharides prevent Wistar rat breast carcinogenesis

Gamal-Eldein F Abd-Ellatef et al, investigated on the isolation and function of the polysaccharides derived from different algal species, which revealed multiple biological activities such as antioxidant and antitumor activities by using in vitro bioassays on human breast cancer cell line (MCF-7) and an in vivo animal model of breast carcinogenesis.

Breast Cancer (Dove Med Press). 2017 Feb 27;9:67-83. doi: 10.2147/BCTT.S12516

Mammary Cancer in 7,12-Dimethylbenz(a)anthracene-Induced Wister Rats by Asymmetrical Temperature Distribution Analysis

S. P. Angeline Kirubha et al investigated that animal surface temperature profile captured using infrared camera is helpful for the assessment of physiological responses associated with the regulation of body temperature. Temperature difference between the tumor induced lower right and left side of flank region was significant (with P value < 0 . 0 0 1), whereas in the abdomen and shoulder there was no significant difference in temperature between right and left sides.. Temperature reduction was observed in the tumor induced region after the seventh day of carcinogen induction.

Volume 2012 (2012), Article ID 786417, 11 pages

Induction of experimental mammary carcinogenesis in rats with 7,12-dimethylbenz(a)anthracene

Alfredo Carlos S. D. Barros et al, investigated that the when 7,12-dimethylbenz(a)anthracene (DMBA) intragastrically by gavage produces mammary gland tumor after the 8 and 13 weeks. At the end of the experiment, the animals were sacrificed, and the mammary tumors were measured and weighed. Tumor fragments were analyzed using light microscopy.

Rev. Hosp. Clin. vol.59 no.5 São Paulo 2004

Protective effect of Operculina turpethum against 7,12-dimethyl benz(a)anthracene induced to breast cancer in experimental rats.

Anbuselvam C et al, investigated the Reactive oxygen species (ROS) directly or indirectly involves in multistage process of carcinogenesis. Antioxidant activity of methanolic extract of Operculina turpethum stems (MEOT) on 7,12 dimethylbenz(a)anthracene (DMBA) induced breast cancer.

A significant ($P<0.05$) increase in the tumour weight was observed in the breast of DMBA group and the breast tumour weight decreased significantly ($P<0.05$) in the DMBA+MEOT groups. Oral administration of MEOT remarkably reduced the lipid peroxidation activity and increased the antioxidants level in drug treated animals and decreased the tumour weight significantly ($P<0.05$).

Chem Biol Interact. 2007 Jul 20;168(3):229-36. Epub 2007 Apr 22.

Cholestyramine promotes 7,12-dimethylbenzanthracene induced mammary cancer in Wistar rats.

M. F. Melhem et al carried out research on the promotion of 7,12-dimethylbenzanthracene (DMBA) induced mammary cancer in Wistar rats by a 4% cholestyramine (CHST) diet. In rats treated with DMBA and fed a 4% CHST diet, the incidence of malignant tumours increased by 5 fold, and the tumour weight by 12 fold. In addition, the serum total lipids, cholesterol esters and triglycerides decreased significantly when compared with rats fed a control diet. These results suggest that CHST diet promotes DMBA induced mammary cancers in Wistar rats.

Br J Cancer. 1987 Jul; 56(1): 45–48.

Black tea and mammary gland carcinogenesis by 7,12-dimethylbenz[a]anthracene in rats fed control or high fat diets.

Rogers AE et al studies suggest that chemopreventive effects of tea or purified extracts of tea have been demonstrated in lung, gastrointestinal tract and skin, mammary gland. Rats fed the HF diet and given 2% tea showed no increase in tumor burden; their tumor burden was significantly lower than in rats fed the HF diet and given water ($P < 0.01$) and was not different from rats fed control diet and given water or tea. In addition, in experiment 3, the number of malignant tumors per tumor-bearing rat was increased by the HF diet in water-drinking rats ($P < 0.01$) but not in tea-drinking rats. Therefore, it appears that tea partially blocked the promotion of DMBA-induced mammary tumorigenesis by the HF diet.

Carcinogenesis. 1998 Jul;19(7):1269-73.

Effects of different animal and vegetable fats fed in rats.

Sylvester PW et al investigated to determine whether diets high in animal or vegetable fat affected mammary tumorigenesis when fed to rats only prior to and

during the initiation phase of carcinogenesis. In conclusion, high dietary intake of lard and beef tallow, but not vegetable fat, fed from weaning until only 1 week after DMBA administration, significantly enhances mammary tumorigenesis in rats.

Cancer Res. 1986 Feb;46(2):757-62.

Chemopreventive effect of Annona muricata in the breast tissues of female albino mice.

J.B. Minari et al was aimed at evaluating the potential chemopreventive effect of an ethanolic extract of Annona muricata leaves on 7,12-dimethylbenzeneanthracene (DMBA)-induced cell proliferation in the breast tissues of female albino mice. This study has shown that the leaf extract of A. muricata could be used as a prophylactic measure against DMBA-induced cell proliferation in the breast tissues of female albino mice.

DOI: 10.1016/j.ejmhg.2014.05.001

Cow ghee versus soybean oil on 7,12-dimethylbenz(a)-anthracene induced mammary carcinogenesis & expression of cyclooxygenase-2 & peroxisome proliferators.

Rita Rani et al investigated the effect of feeding cow ghee versus soybean oil on 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary cancer in rat and expression of cyclooxygenase-2 and peroxisome proliferators activated receptor- γ (PPAR-γ) in mammary gland. Histological analysis of tumours showed that the progression of carcinogenesis was more rapid on soybean oil than on cow ghee. The expression of cyclooxygenase-2 was observed only in DMBA treated rats and it was significantly less on cow ghee than on soybean oil. The expression of PPAR-γ was significantly more on cow ghee than on soybean oil. The effect is mediated by decreased expression of cyclooxygenase-2 and increased expression of PPAR-γ in the former group.

Indian J Med Res. 2011 May; 133(5): 497–503

7,12-dimethylbenzanthracene induced mammary tumours in wistar rats by 'air pouch' technique

B. Arun, et al investigated an 'air pouch' technique of inducing highly localized, transplantable, estrogen-dependent adenocarcinomas of the mammary gland in Wistar rats with 7,12-dimethylbenzanthracene (DMBA). Using this model estrogen receptor status, transplantation and effect of exogenous hormones on tumour growth have been studied.

December 1984Volume 25, Issue 2, Pages 187–19

Inhibition of 7,12-dimethylbenzanthracene-induced rat mammary tumor growth by 2,3,7,8-tetrachlorodibenzo-p-dioxin

M. Holcomb et al carried out a research on 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related compounds inhibit diverse estrogen-induced responses in the rodent uterus and human breast cancer cells. The effects of a single non-toxic dose of TCDD (10 μg/kg) on the development of mammary tumors was investigated in female Sprague-Dawley rats, the mean tumor volumes decreased from 89.7 ± 53 mm3 to 24.9 ± 28.5 mm3. Moreover, these results demonstrate the antitumorigenic activity of TCDD in female Sprague-Dawley rats.

July 15, 1994Volume 82, Issue 1, Pages 43–47

Enhancement of pro-oxidant effect of 7,12-dimethylbenz (a) anthracene (DMBA) in rats by pre-exposure to restraint stress

Muqbil I et al designed to assess the effect of immobilization stress on liver toxicity induced by topical as well as oral administration of 7,12-dimethyl benz(a)anthracene (DMBA) in Swiss Albino rats. Biochemical measurements were carried out on liver tissues and serum/plasma of control and treated animals. The results of the present study indicate that immobilization stress markedly enhances DMBA induced alteration of liver and circulatory antioxidant status of the rats irrespective of the mode of DMBA administration though with a predominant effect on orally infused DMBA.

Cancer Lett. 2006 Aug 28;240(2):213-20. Epub 2005 Nov 2.

Experimentally induced mammary tumors in rats

Jose Russol et al carried out research on the multiple experimental animal models employed for analyzing the various aspects of mammary carcinogenesis. Using the DMBA rat mammary model, we have been able to demonstrate that the carcinogen acts on the intermediate cell of the terminal end bud (TEB), and that this structure is the one that evolves to intraductal proliferation, carcinoma in situ, and invasive carcinoma. There are several factors that regulate the susceptibility of the TEB; some of them are: a) topographic location of the mammary gland, b) age of the animal, and c) reproductive history.

Breast Cancer Res Treat. 1996;39(1):7-20.

Suppression of 7,12-Dimethylbenz(α) Anthracene-Induced Breast Carcinoma by Coumarin in the Rat

Feuer G et al carried out a research on the effect of oral administration of coumarin on the induction of breast tumors brought about by DMBA has been studied in female Wistar rats. Coumarin given after DMBA elicited no effect on the carcinogenic potency of DMBA. In contrast to the action on mammary tumorigenesis, coumarin provided no protection against hemopoietic and adrenocortical necrosis brought about by DMBA.

Oncology 1976;33:35–39 (DOI:10.1159/000225098)

Activity of Avocado:

Effect of Semisolid Formulation of Persea Americana Mill (Avocado) Oil on Wound Healing in Rats

Ana Paula de Oliveira et al carried out the study to evaluate the wound-healing activity of a semisolid formulation of avocado oil, SSFAO 50%, or avocado oil in natura, on incisional and excisional cutaneous wound models in Wistar rats. An additional objective was to quantify the fatty acids present in avocado oil. Anti-inflammatory activity, increase in density of collagen, and tensile strength were observed in SSFAO 50% or avocado oil groups. Results show that avocado oil is a rich source of oleic acid and contains essential fatty acids, avocado oil can promote increased collagen synthesis and decreased numbers of inflammatory cells during the wound-healing process.

Evid Based Complement Alternat Med. 2013; 2013: 472382

Inhibition of prostate cancer cell growth by an avocado extract

Qing-Yi Lu et al carried out an research on acetone extract of avocado containing these

carotenoids and tocopherols was shown to inhibit the growth of both androgen-dependent (LNCaP) and androgen-independent (PC-3) prostate cancer cell lines in vitro. Incubation of PC-3 cells with the avocado extract led to G2/M cell cycle arrest accompanied by an increase in p27 protein expression. Lutein alone did not reproduce the effects of the avocado extract on cancer cell proliferation..

Journal of Nutritional Biochemistry 16 (2005) 23 – 30

Apoptosis of human oral cancer cell lines by avocado extracts via a ROS-mediated mechanism.

Ding H, et al investigated that avocados have a high content of phytochemicals with potential chemo preventive activity. The phytochemicals extracted from avocado induced apoptosis in cancer but not normal, human oral epithelial cell lines. In this study treatment of human oral cancer cell lines containing high levels of reactive oxygen (ROS) with D003 increased ROS levels twofold to threefold and induced apoptosis.

2009;61(3):348-56.

Effect of the Water Extracts of Avocado Fruit and Cherimoya Leaf on Human Cancer Cell Lines and Vicia Faba Root Tip Cells

Noha S. Khalifa et al investigated the effect of the water extract of Persea americana Mill. (avocado fruit) and Annona cherimolia Mill (cherimoya leaf) on living cells. The antiproliferative properties of avocado fruit water extract (AFWE) and cherimoya leaf water extract (CLWE) were determined using four human cancer cell lines: lung (A549), liver (HepG-2), colon (HT-29) and breast (MCF-7). The prophase cell percentage was linearly increasing with the applied concentration without micronuclei formation in avocado treatment. Results indicate that avocado and cherimoya extracts were highly cytotoxic and mitodepressive on cancer and plant cells.

Journal of Agricultural Science; Vol. 5, No. 7; 2013

Tumor-promoting and tumor-protective effects of high-fat diets on chemically induced mammary cancer in rats.

Zusman I et al investigated the effects of different dietary fats on experimental rat mammary lumorigenesis induced by 9,10-dimethyl-1, 2-benzanthracene (DMBA). The olive diet was associated with a significant reduction in the tumorigenic effect of

DMBA: tumor incidence decreased to 30%, as compared to 44%-55% in the other dietary groups studied ($p < 0.05$). Malignant mammary tissue exhibited higher values than benign tissue for all the argyrophilic-nucleolar-organizer region parameters measured. The tumor-associated protein p53 was accumulated to high levels in the blood of tumor-bearing rats, but not in that of the non tumor-bearing rats. Anticancer Res. 1997 Jan-Feb;17(1A):349-56.

Hass Avocado Composition and Potential Health Effects

Mark L et al investigated that the avocado oil consists of 71% monounsaturated fatty acids (MUFA), 13% polyunsaturated fatty acids (PUFA), and 16% saturated fatty acids (SFA), which helps to promote healthy blood lipid profiles and enhance the bioavailability of fat soluble vitamins and phytochemicals from the avocado or other fruits and vegetables, naturally low in fat, which are consumed with avocados.

Crit Rev Food Sci Nutr. 2013 May; 53(7): 738–750.

Activity of Aloe vera:

Clear Evidence of Carcinogenic Activity by a Whole-Leaf Extract of Aloe barbadensis Miller (Aloe vera) in F344/N Rats

Mary D. Boudreau et al investigated on drinking water exposure of F344/N rats and B6C3F1 mice to an Aloe vera whole-leaf extract (1, 2, and 3%) for 13 weeks resulted in goblet cell hyperplasia of the large intestine in both species. Incidences of adenomas and/or carcinomas of the ileo-cecal and cecal-colic junction, cecum, and ascending and transverse colon were significantly higher than controls in male and female rats in the 1 and 1.5% dose groups. Increased incidences of mucosa hyperplasia of the large intestine were observed in F344/N rats, and increased incidences of goblet cell hyperplasia of the large intestine occurred in B6C3F1 mice. These results indicate that Aloe vera whole-leaf extract is an intestinal irritant in F344/N rats and B6C3F1 mice and a carcinogen of the large intestine in F344/N rats.

Toxicol Sci. 2013 Jan; 131(1): 26–39

Aloe vera non-decolorized whole leaf extract-induced large intestinal tumors in F344 rats

Pandiri AR et al investigated on NTP/NCTR demonstrated a dose-dependent increase in large intestinal tumors in F344 rats chronically exposed to Aloe barbadensis Miller (Aloe vera) non-decolorized whole leaf extract (AVNWLE) in drinking water. The commonly mutated genes (Kras, Ctnnb1, and Tp53) and altered signaling pathways (MAPK, WNT, and TGF-β) important in hCRC were evaluated within AVNWLE-induced large intestinal tumors. In conclusion, the AVNWLE-induced large intestinal tumors in F344 rats share several similarities with hCRC at the morphological and molecular levels.

Toxicol Pathol. 2011 Dec;39(7):1065-74. doi: 10.1177/0192623311422081.

Safety of purified decolorized (low anthraquinone) whole leaf Aloe vera (L) Burm. f. juice in F344 rats

Shao A, et al investigated on decolorized (purified, low anthraquinone) whole leaf Aloe vera (L.) Burm. f. juice was administered at concentrations of 0%, 0.5%, 1% and 2% in the drinking water of F344Du rats. Similar concentrations of non-decolorized (unpurified, high anthraquinone) Aloe vera extracts tested in other studies have resulted in an increased incidence and severity of diarrhea and colon adenomas and carcinomas. The results of this study supports the assertion that the high levels of anthraquinone present in orally administered, non-purified whole leaf Aloe vera extract may be responsible for the adverse effects observed on the colon.

Food Chem Toxicol. 2013 Jul;57:21-31. doi: 10.1016/j.fct.2013.03.002

The effects of six weeks endurance training and Aloe Vera on COX-2 and VEGF levels in mice with breast cancer

Alireza Barari investigated on the effects of the effects of six weeks endurance training and Aloe Vera on cyclooxygenase 2 (COX-2) and VEGF levels in mice with breast cancer. K-S test to determine the normality of the data and analysis of variance for repeated measures and Tukey test was used to analyze the data. A significant difference in the $p<0.05$ accepted.

The Review on Properties of Aloe Vera in Healing of Cutaneous Wounds

Seyyed Abbas Hashemi et al carried research on natural substance plays crucial role as complementary medicine. Various studies reported that aloe vera has useful effects on wounds especially cutaneous wounds healing. Therefore in the current review, we examined the effect of aloe vera on cutaneous wound healing and concluded that although aloe vera improves the wound healing.

Volume 2015 (2015), Article ID 714216, 6 pages

Anti inflammatory activity of extracts from Aloe vera gel

Beatriz Vázquez, et al investigated the effects of aqueous, chloroform, and ethanol extracts of Aloe vera gel on carrageenan-induced edema in the rat paw, and neutrophil migration into the peritoneal cavity stimulated by carrageenan. Also studied the capacity of the aqueous extract to inhibit cyclooxygenase activity. The aqueous extract inhibited prostaglandin E2 production from arachidonic acid. These results demonstrated that the extracts of Aloe vera gel have antiinflammatory activity and suggested its inhibitory action on the arachidonic acid pathway via cyclooxygenase.

Volume 55, Issue 1, December 1996, Pages 69-75

Activity of Ashwagandha:

An Overview on Ashwagandha: A Rasayana (Rejuvenator) of Ayurveda

Narendra Singh, et al carried out a research on pretreatment of Withania somnifera (WS) showed significance protection against stress induced gastric ulcers. WS have anti-tumor effect on Chinese Hamster Ovary (CHO) cell carcinoma. It was also found effective against urethane induced lung-adenoma in mice. It was also found useful in neurodegenerative diseases such as Parkinson's, Huntington's and Alzeimer's diseases. It has GABA mimetic effect and was shown to promote formation of dendrites. It has anxiolytic effect and improves energy levels and mitochondrial health.

Afr J Tradit Complement Altern Med. 2011; 8(5 Suppl): 208–213

Effects of Ashwagandha (roots of Withania somnifera) on neurodegenerative diseases

Kuboyama T, et al investigated on Neurodegenerative diseases commonly induce irreversible destruction of central nervous system (CNS) neuronal networks, resulting in permanent functional impairments. Ashwagandha (roots of Withania somnifera Dunal) is used in traditional Indian medicine (Ayurveda) for general debility, consumption, nervous exhaustion, insomnia, and loss of memory. In this review, the various effects and mechanisms of Ashwagandha extracts and related compounds on in vitro and in vivo models of neurodegenerative diseases such as Alzheimer's disease and spinal cord injury.

Biol Pharm Bull. 2014;37(6):892-7

Water Extract of Ashwagandha Leaves Has Anticancer Activity

Renu Wadhwa ,et al carried research on anticancer activity in the water extract of Ashwagandha leaves (ASH-WEX) was detected by in vitro and in vivo assays. It was reported that the ASH-WEX is cytotoxic to cancer cells selectively, and causes tumor suppression in vivo. Its active anticancer component was identified as triethylene glycol (TEG). Molecular

analysis revealed activation of tumor suppressor proteins p53 and pRB by ASH-WEX and TEG in cancer cells. In contrast to the hypophosphorylation of pRB, decrease in cyclin B1 and increase in cyclin D1 in ASH-WEX and TEG-treated cancer cells (undergoing growth arrest), normal cells showed increase in pRB phosphorylation and cyclin B1, and decrease in cyclin D1 (signifying their cell cycle progression).It was found that the MMP-3 and MMP-9 that regulate metastasis were down regulated in ASH-WEX and TEG-treated cancer cells; normal cells remained unaffected.

October 10, 2013 https://doi.org/10.1371/journal.pone.0077189

Selective Killing of Cancer Cells by Ashwagandha Leaf Extract and Its Component Withanone Involves ROS Signaling

Nashi Widodo, et al investigated the randomized ribozyme library was introduced into cancer cells prior to the treatment with i-Extract. Gene targets of the selected ribozymes were analyzed by bioinformatics and pathway analyses. Fifteen gene-targets were identified and were investigated for their role in specific cancer cell killing activity of i-Extract and its two major components (Withaferin A and Withanone) by undertaking the shRNA-mediated gene silencing approach. The selected gene-targets revealed the involvement of p53, apoptosis and insulin/IGF signaling pathways linked to the ROS signaling. It was examined the involvement of ROS-signaling components and demonstrate that the selective killing of cancer cells is mediated by induction of oxidative stress.

October 21, 2010https://doi.org/10.1371/journal.pone.0013536

Effect of an Extract of Withania somnifera Root on Estrogen Receptor-positive Mammary Carcinomas

KAMEL F. KHAZAL, et al investigated the chemopreventive activity of an extract of Withania somnifera (WS) roots was examined in female Sprague-Dawley rats that received the mammary carcinogen methylnitrosourea (MNU). Labeling indices for Ki67 and proliferating cell nuclear antigen (PCNA) markers in cancers of the treated group were 42% and 38% lower, respectively, than those of the corresponding indices

for the control group. These results indicate that the root extract significantly reduced the rate of cell division in the mammary tumors.

Anticancer Res. 2013 Apr; 33(4): 1519–1523.

Effect of Withania somnifera root extract on spontaneous estrogen receptor-negative mammary cancer in MMTV/Neu mice.

Khazal KF, et al carried out a research on the cancer-preventive activity of an extract of Withania somnifera (WS) roots was examined in female transgenic (MMTV/Neu) mice. The average weights of the carcinomas were 2.36 g for mice in the treated group and 2.63 g for the controls, a difference of 10%. Labeling indices for Ki67 and proliferating cell nuclear antigen marker in mammary carcinomas of the treated group were 35% and 30% lower, respectively, than those of the corresponding control group. Expression of the chemokine was reduced by 50%. These results indicate that the root extract reduced the number of mammary carcinomas that developed and reduced the rate of cell division in the carcinomas.

Anticancer Res. 2014 Nov;34(11):6327-32

In Vitro Anticancer Activity of the Root, Stem and Leaves of Withania Somnifera against Various Human Cancer Cell Lines

B. Yadav, et evaluated in vitro cytotoxicity in 50% ethanol extract of root, stem and leaves of Withania Somnifera against five human cancer cell lines of four different tissues i.e. PC-3, DU-145 (prostrate), HCT-15 (colon), A-549 (lung) and IMR-32 (neuroblastoma). Ethanol extract of leaves obtained from treatments T2, T3, T4 and T5 showed strong activity against PC-3 and HCT-15 with 80-98% growth inhibition, while the 50% ethanol extract of leaves from T1 treatment showed a minimum of 39% and T3 treatment showed a maximum of 98% growth inhibition against HCT-15. This investigation is the first report of the anticancer activity in various parts of Withania Somnifera.

Indian J Pharm Sci. 2010 Sep-Oct; 72(5): 659–663.

Withania somnifera Root Extract Inhibits Mammary Cancer Metastasis and Epithelial to Mesenchymal Transition

Zhen Yang, et al Withania somnifera root extracts (WRE) have anti-proliferative activity and the active component, Withaferin A, inhibits the pro-metastatic protein, vimentin. Vimentin is an intermediate filament protein and is part of the epithelial to mesenchymal transition (EMT) program to promote metastasis. It was determined whether WRE standardized to Withaferin A (sWRE) possesses anti-metastatic activity and whether it inhibits cancer motility via inhibition of vimentin and the EMT program. This sWRE formulation inhibited breast cancer cell motility and invasion. These studies were taken into a human xenograft and mouse mammary carcinoma model. Taken together, these data support the hypothesis that low concentrations of sWRE inhibit cancer metastasis potentially through EMT inhibition.

PLoS One. 2013; 8(9): e75069. Published online 2013 Sep 12. doi: 10.1371/journal.pone.0075069

5.1 **Introduction to Herbal Plants**

Persea Americana Mill [90]

Description

A rapidly-growing tree or shrub from Mexico and Central America, avocado is well-known for its edible, green-fleshed fruits.

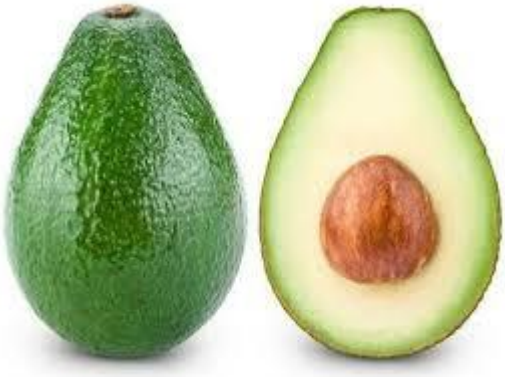
Avocado pear, alligator pear, midshipman's butter, vegetable marrow, avocatier (French), aguacate (Spanish), avokado (Swedish), Butter Fruit

The avocado belongs to the laurel family (Lauraceae), which includes many aromatic trees and shrubs. The common name comes from the Nahuatl (a Mexican language) word *ahuácatl*, meaning testicle, and refers to the shape of the fruits, an association which led to their being considered a fertility food.

Avocados have been cultivated for their highly nutritious fruits since about 8,000 BC, and there is evidence that they were eaten as a wild fruit before then. The oldest archaeological record is from Coxcatlan, Mexico, from about 10,000 BC.

Avocado Leaves[92]

Avocado Flower[92]



Avocado Fruit[92]

Taxonomy[91]

Kingdom	Plantae
(unranked)	Angiosperms
(unranked)	Magnoliids
Order	Laurales
Family	Lauraceae
Genus	Persea
Species	P. americana
Binomial name	Persea americana Mill.

Active Ingredient and Substances [90]:

The leaves contain essential oil (with estragole, caryophyllene and eugenol), flavonoids (afzelin, cynaroside, luteolin, quercetin), tannins and procyanidins.

The avocado fruit is considered one the most nutritious of all fruits and very high in calories (220 kcal per 100 g). The fruit contains a fatty oil based on linoleic acid, linolenic acid, palmitic acid, stearic acid and other fatty acids, both saturated and unsaturated.

Furthermore, it contains phenols (caffeic acid, chlorogenic acid, p-coumaric acid), alpha-carotene, beta-carotene, folic acid, pantothenic acid, fructose, glucose, sucrose, fibers, amino acids and small amounts of coenzyme Q10. The avocado pulp contains more protein than any other fruit.

The fruit is loaded with vitamin B1 (thiamine), B2 (riboflavin), B3 (niacin), B6, C, D, and E (tocopherols, tocotrienols) and minerals such as calcium, sodium, potassium, zinc, iron, phosphorus, copper, manganese and magnesium.

Avocado oil containing 20-35% saturated fatty acids (palmitic acid, myristic acid, and stearic acid), 70 to 85% monounsaturated fatty acids (oleic and palmitoleic acids) and 10-15% polyunsaturated fatty acids (linoleic and linolenic acids).

Application of the Leaves, Bark and Seeds of Avocado [90]**:**

1. Remedy for High Cholesterol
2. Atherosclerosis, Angina Pectoris and Alzheimer's Disease
3. Hypertension and coronary heart disease
4. Good for the Digestion and Blood Sugar Balance

5. Skincare
6. Avocado Oil Uses in Aromatherapy, Cosmetics and Skincare
7. Pregnancy, Birth or Puerpuerium Disorders
8. Aphrodisiac
9. Diarrhoea, bloating and flatulence,dysentry
10. Remedy for coughs and gout
11. Cleanse the liver
12. Inhibit herpes simplex virus type I and II, which causes cold sores (I) and genital herpes (II)
13. Antibacterial and antifungal properties
14. Antiviral and anti-inflammatory effects

5.2 *Aloe vera (L.) Burm.f.*

Description[93]

Aloe vera is a stemless or very short-stemmed plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (35 in) tall, each flower being pendulous, with a yellow tubular corolla 2–3 cm (0.8–1.2 in) long. Like other *Aloe* species, *Aloe vera* forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil.

Aloe vera leaves contain phytochemicals under study for possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, other anthraquinones, such as emodin and various lectins

Taxonomy[93]

Kingdom	Plantae
Clade	Angiosperms
Clade	Monocots
Order	Asparagales
Family	Asphodelaceae
Subfamily	Asphodeloideae
Genus	Aloe
Species	A. vera
Binomial name	Aloe vera (L.) Burm.f.

Active components with its properties[94]:

Aloe vera contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids.

Vitamins: It contains vitamins A (beta-carotene), C and E, which are antioxidants. It also contains vitamin B12, folic acid, and choline. Antioxidant neutralizes free radicals.

Enzymes: It contains 8 enzymes: aliiase, alkaline phosphatase, amylase, bradykinase, carboxypeptidase, catalase, cellulase, lipase, and peroxidase. Bradykinase helps to reduce excessive inflammation when applied to the skin topically, while others help in the breakdown of sugars and fats.

Minerals: It provides calcium, chromium, copper, selenium, magnesium, manganese, potassium, sodium and zinc. They are essential for the proper functioning of various enzyme systems in different metabolic pathways and few are antioxidants.

Sugars: It provides monosaccharides (glucose and fructose) and polysaccharides: (glucomannans/polymannose). These are derived from the mucilage layer of the plant and are known as mucopolysaccharides. The most prominent monosaccharide is mannose-6-phosphate, and the most common polysaccharides are called glucomannans [beta-(1,4)-acetylated mannan]. Acemannan, a prominent glucomannan has also been found. Recently, a glycoprotein with antiallergic properties, called alprogen and novel anti-inflammatory compound, C-glucosyl chromone, has been isolated from Aloe vera gel.

Anthraquinones: It provides 12 anthraquinones, which are phenolic compounds traditionally known as laxatives. Aloin and emodin act as analgesics, antibacterials and antivirals.

Fatty acids: It provides 4 plant steroids; cholesterol, campesterol, β-sisosterol and lupeol. All these have anti-inflammatory action and lupeol also possesses antiseptic and analgesic properties.

Hormones: Auxins and gibberellins that help in wound healing and have anti-inflammatory action.

Others: It provides 20 of the 22 human required amino acids and 7 of the 8 essential amino acids. It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties. Lignin, an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients into the skin. Saponins that are the soapy substances form about 3% of the gel and have cleansing and antiseptic properties.

Application of aloe vera[94]:

1. Antioxidant and antibacterial properties
2. Antitumor activity
3. Accelerates the healing of burns
4. Reduces dental plaque
5. Helps treat mouth ulcers, or canker sores
6. Reduces constipation
7. Effective against other digestive disorders, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD)
8. Prevent wrinkles
9. Lowers blood sugar levels in diabetics

10. Anti-inflammatory action

11. Effects on the immune system

12. Moisturizing and anti-aging effect

13. Antiseptic effect

5.3 Withania somnifera[95]:

Description:

Ashwagandha in Sanskrit means "smelling like a horse ", Probably originating from the odor of its fresh root which resembles that of a sweaty horse.

Ashwagandha root is a herb of the ages. It is the 'ginseng' of Ayurvedic medicine. Ancient Ayurvedic texts including the Charaka and Sushruta Samhitas praise Ashwagandha as a tonic particularly for emaciation in people of all ages of both men and women. The species name somnifera means "sleep-inducing" in Latin.

This species is a short, tender perennial shrub growing 35–75 cm (14–30 in) tall. Tomentose branches extend radially from a central stem. Leaves are dull green, elliptic, usually up to 10–12 cm (4 to 5 in) long. The flowers are small, green and bell-shaped. The ripe fruit is orange-red.

FLOWER

LEAVES

FRUIT

ROOTS

Vernacular names[96]

Ajagandha, Amangura, Amukkirag, Asan, Asana, Asgand, Asgandh, Asgandha, Ashagandha, Ashvagandha, Ashwaganda, Ashwanga, Asoda, Asundha, Asvagandha, Aswagandha, Avarada, Ayurvedic Ginseng, Cerise d'Hiver, Clustered Wintercherry, Ghoda Asoda

Taxonomy[97]

Kingdom	**Plantae**
(unranked)	**Angiosperms**
(unranked)	**Eudicots**
(unranked)	**Asterids**
Order	**Solanales**
Family	**Solanaceae**
Genus	**Withania**
Species	**W. somnifera**
Binomial name	**Withania somnifera**

Active components with its properties[97]**:**

Ashwagandha root contains flavonoids and many active ingredients of the withanolide class. The distinctive earthy odor and flavor of Ashwagandha is due to the presence of certain steroidal lactones or Withanolides. These molecules which are steroidal in nature are believed to account for the multiple medicinal applications of Ashwagandha.

Other major Alkaloids: Somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropino, pseudotropine, choline, cuscohygrine, isolettetierine, anaferine, anahydrine, 3-alpha-gloyloxy tropane, etc.

The ashwagandha consists of alkaloids and steroidal lactones. Withanine is the main alkaloid, where as somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo tropine, choline, cuscohygrine, isopelletierine, anaferine, anahydrine. Acyl steryl glucosides namely sitoindoside-v11 and sitoindoside-v111 have been isolated from the roots. The leaves contain steroidal lactones called as withanolides. They have steroidal nucleus, carbon chain and lactone ring. WIthaferin and withaferin-A are some of the withanolides. It also contains alcohols like somnitol and somnirol, withanic acid, phytosterol, ipuranol and cerotic acid, oleic acid, palmitic acid and stearic acid.

Uses of Ashwagandha[97] **:**

1. Sedative
2. Hypnotic
3. Hypotensive
4. Respiratory actions
5. Stimulant
6. Treatment of bradycardia
7. Immuno-modulatory agent
8. Anti-stress agent
9. Treatment of rheumatism
10. Treatment of gout
11. Hypertension
12. Nerve tonic
13. Used for skin diseases
14. Prevents degenerative changes in the bones in arthritic conditions.
15. Sex stimulant

16. Rejuvenator
17. Strength and vigour promoting drug in geriatrics (old people)
18. Leaf extract shows activity against Staphylococcus aureus and Ranikhet virus.
19. It is a natural nutrient for insomnia
20. It is good hypnotic in Alcoholism.
21. It stimulates thyroid activity.
22. Enhances anti-peroxidation of liver.
23. Decrease high cholesterol levels
24. It balances the mind (Prana Vata).
25. Cancer treatment and prevention
26. It increases the quality and quantity of Ojas, the master coordinator between the body and consciousness.
27. Antioxidant properties

EXPERIMENTAL ANIMAL MODEL FOR CANCER STUDIES

Cancer Model [98]

- Animals in which **cancers occur spontaneously** without any alteration of the animal's genes or initiation of cancer by chemical treatments.
- Animals whose **genes are altered** so that they develop spontaneous tumors of the same types and with similar properties as the tumors which those altered genes cause in humans.
- Animals that develop spontaneous tumors if they are **exposed to environmental factors**, such as chemicals or radiation.
- Animals whose natural, unaltered genetic makeup permits researchers to **identify the genes** that generate susceptibility to cancer development.
- The utility of the models varies by species, by available research reagents and tools to support the studies, and by similarity to the human situation. The most commonly used animal cancer models are rodents - mice and rats. Other cancer models include hamsters, rabbits, dogs, cats, livestock, and fish.

Rodent cancer models[99]

Mouse cancer models are well known and are frequently used as models for cancer research. Mouse models have revolutionized our ability to study gene and protein function in vivo and to better understand their molecular pathways and mechanisms. The most common rodent cancer models are xenografts and chemically or genetically induced cancers.

Rodent xenograft cancer model[99]:

In the xenograft cancer model, human or animal cancer cells are transplanted either under the skin (ectopic) or into the organ of tumor origin (orthotopic) using immunocompromised rodents. Xenograft animal cancer models are a relatively inexpensive method for generating in vivo tumors using human and animal cancer cell lines.

The major disadvantages of the xenograft rodent cancer models that limit the rapid translation of research to the human clinic include; effectiveness of specific anticancer drugs toward only certain cancer tumors, the superficial vascularization of xenograft tumors, and the lack of stroma-tumor interactions. The major limitation of xenograft cancer models is that the mice and rats used have compromised immune systems, so do not represent the behavior of naturally occurring cancers in humans.

Chemically induced rodent models of cancer[99]:

Chemically induced rodent models of cancer are developed by exposure to carcinogens, eg, DMBA(7,12-Dimethylbenz(a)anthracene), N-Methylnitrosourea (MNU), N-butyl-N-(4-hydroxybutyl) nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, N-ethyl-N-nitrosourea, azoxymethane(AOM) , benzopyrene, urethane, and asbestos fibers. Chemically induced cancer rodenet models help in the study of the complex traits of cancer, but require high-throughput sequencing to identify the mutations, making the method laborious and time-consuming.

Genetically engineered mouse models of cancer[99]:

- Spontaneous Method

Induction of various oncogenes or suppression of tumor suppressor genes, leading to the development of spontaneous cancers. The most commonly used systems are Cre-Lox,tetracycline-dependent promoter regulation, and Flp-mediated site-specific and spontaneous recombination methods.

- Genetically modified mice are created by microinjection of DNA in the pronuclei of fertilized zygotes and the transgene is integrated into the genome.
- Transgenic mice (SK Gupta)

Cancer is known to be a disease of genome and a large number of human cancers arise from mutation in one or more oncogene or tumor suppressor gene. Therefore, inactivation of a particular gene within specific tissues of adult mouse. The genetically engineered mouse may serve both as a model of disease as well as a model for possible gene therapy. Such mice can be generated either by pronuclear injection of DNA or by gene targeting

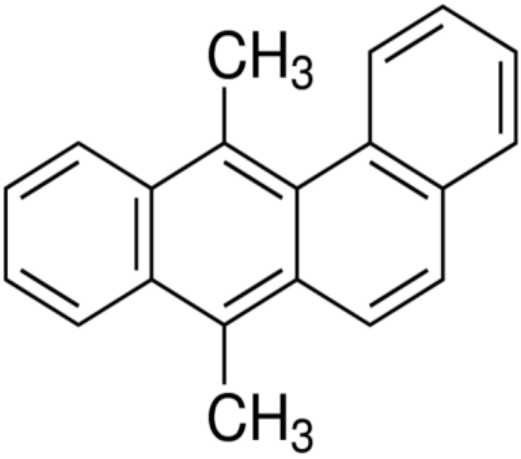
- Nude Mouse Model

Nude mice have been widely used to test the tumorigenicity of cells or for testing of anticancer drugs. These mice are immunologically incompetent because of absence of thymus. Neither show mitotic response nor generate cytotoxic effector cell. Lack of helper T cells and suppressor T cells.

7,12-Dimethylbenz[a]anthracene (DMBA)

DMBA is a polycyclic aromatic hydrocarbon that is widely used as a chemical carcinogen for induction of mammary tumor model. It is probably the best studied polycyclic aromatic hydrocarbon available, besides benzo[a]pyrene[100].

DMBA is an immunosuppressant and a strong mammary tumour specific carcinogen. That is why, it is widely used worldwide for studying cancer. The mechanism of action of DMBA is basically that of a tumor i`nitiator[101].



Structure of 7,12-Dimethylbenz[a]anthracene

IUPAC Name: 7,12-Dimethylbenz[a]anthracene
Synonyms: 9,10-Dimethyl-1,2-benzanthracene
1,4-Dimethyl-2,3-benzophenanthrene

Mechanism of Carcinogenesis[102, 103]:

DMBA is metabolized to 3,4-diol metabolite by cytochrome P450. This metabolite of DMBA is considered as the procarcinogen that is finally metabolized by cytochrome P450 to 1,2-epoxide-3,4-diol-DMBA which is the ultimate carcinogen and forms adducts in DNA. These adducts in the DNA lead to mutations which is a essential requirement for the development of tumors. The cytochrome P450 responsible for this metabolic activation for decades was assumed to be CYP1A1 Expressed or induced CYP1A1 was capable of activating DMBA to the procarcinogen 3,4-diol DMBA**.** That CYP1A1 was crucial *in vivo* for the development of cancers was then concluded from these, often *in vitro* studies. However, later on it was discovered that the regulation of CYP1B1 expression is similar to the that of CYP1A1, and the observed *in vivo* effects could have been equally attributed to CYP1B1. Before 1994, the existence of CYP1B1 was not known. A study revealed that *in vivo* CYP1B1 is the predominant enzyme in the metabolic activation of DMBA to carcinogenic metabolites and not CYP1A1. CYP1B1 is expressed in human lung, can play an important role in the metabolic activation tobacco smoke components like polycyclic aromatic hydrocarbons to carcinogenic metabolites.

Metabolic activation of polycyclic aromatic hydrocarbons and the formation of DNA adducts are a prerequisite of chemical carcinogenesis, but additional factors are required to transform these adducts into mutations and subsequently tumors. Metabolic activation mediated by CYP1B1 is the first step in the pathway of chemical carcinogenesis leading to tumors.

AIM & SCOPE OF WORK

Cancer, being a cause of death for major fraction of population worldwide, is one of the most studied diseases and is being investigated for the development of new technologies and more accurate therapies. Still the currently available therapies for cancer have many lacunae which affect the patient's health severely in the form of side effects. The natural drugs obtained from the medicinal plants provide a better alternative to fight against this devastating disease.

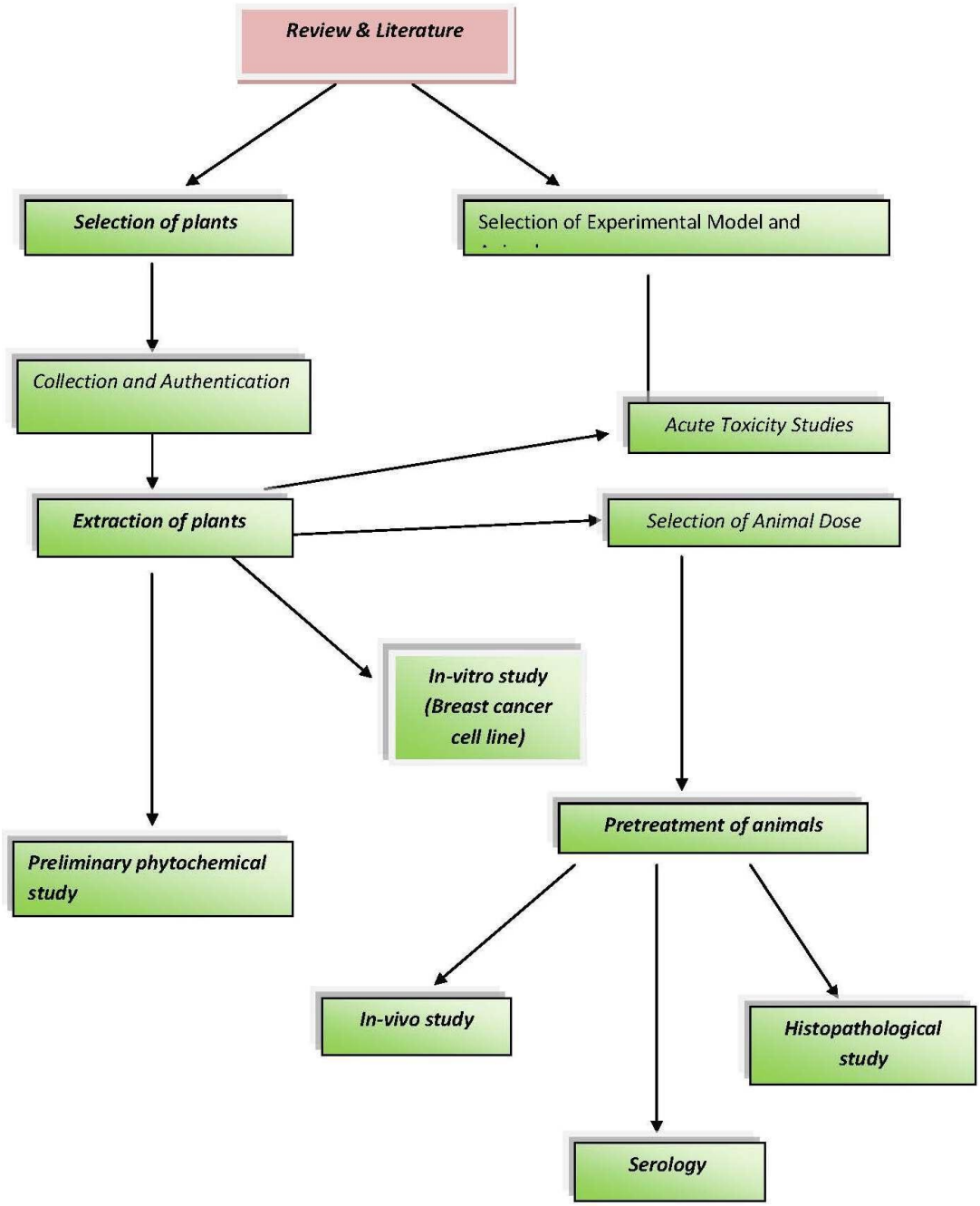
Breast cancer and cancer related diseases have been treated using surgery, chemotherapy, and radiation therapy, or a combination of these. Despite these therapeutic options, cancer remains associated with high mortality. Traditional medicine which involves the use of herbs has been used to treat various types of cancer and this has been found to be effective with minimal or no side effects. It was also highlighted in introductory part the need of alternative system of herbal medicine for treating various ailments.

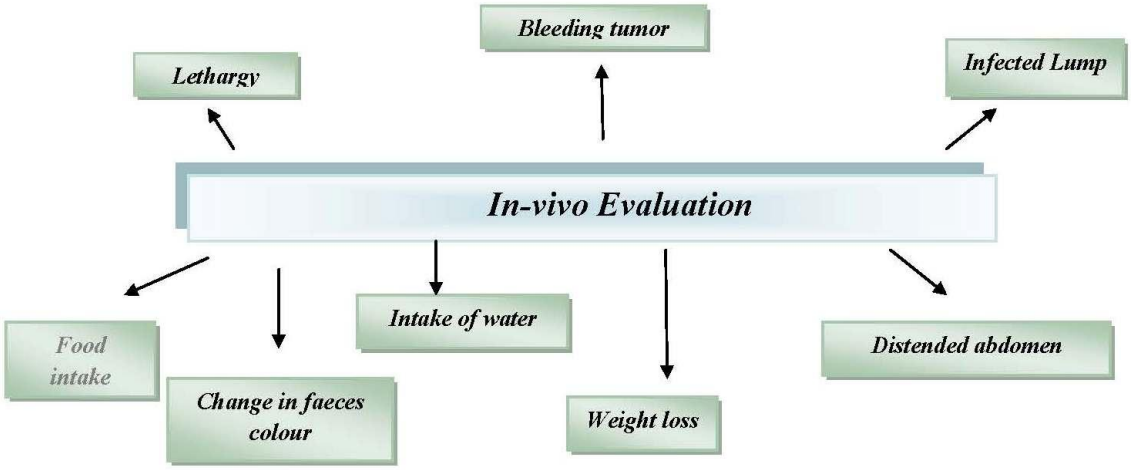
The review of literature shows many uses and different studies for cancer on Avocado, Aloe vera and Ashwagandha.

This research was aimed to evaluate the both in-vitro studies using Breast cancer cell line (MCF-7) & in-vivo studies based on the ethanolic extract of ASVERADO for its anti-neoplasttic activity against 7,12-dimethylbenzeneanthracene (DMBA) induced Mammary gland carcinoma in female Wistar rats. So that it may serve clinically for the management of cancer.

PLAN OF WORK

PLAN OF WORK:

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Lethargy
Infected Lump
In-vivo Evaluation
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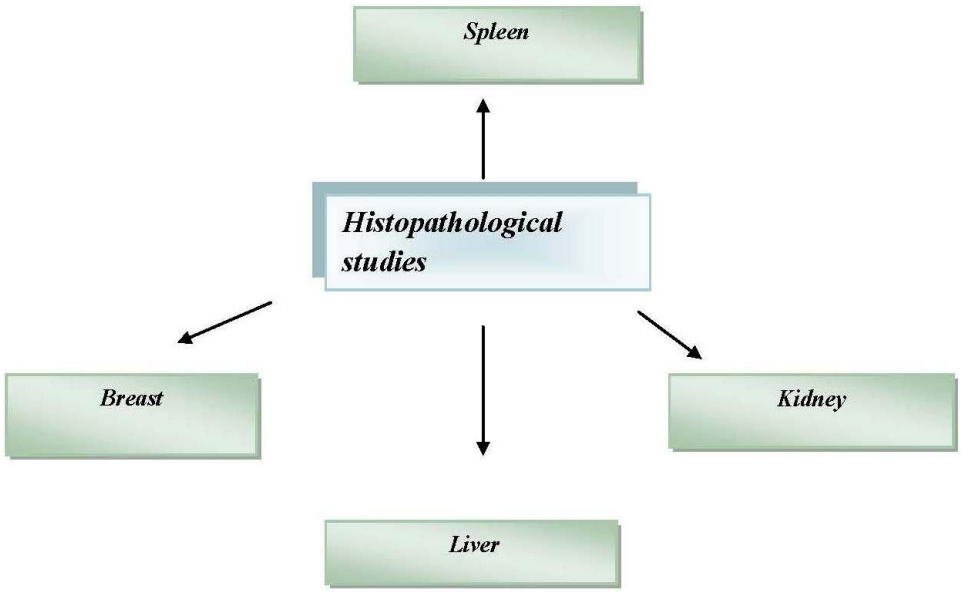
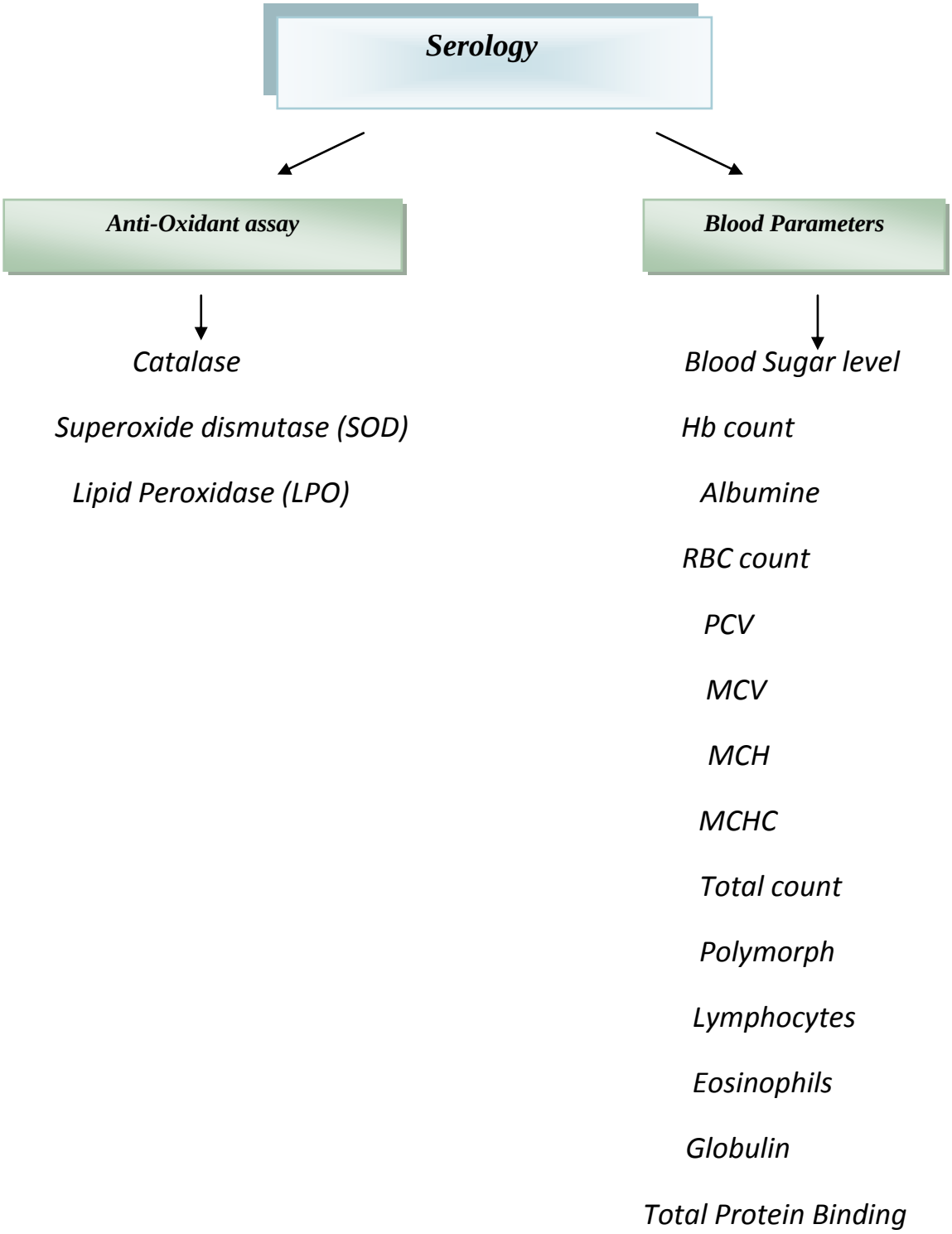
Intake of water
Change in faeces colour
Weight loss
Distended abdomen


Spleen
Histopathological studies
Breast
Kidney
Liver


Serology
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Blood Parameters
Catalase
Superoxide dismutase (SOD)
Lipid Peroxidase (LPO)
Blood Sugar level
Hb count
Albumine
RBC count
PCV
MCV
MCH
MCHC
Total count
Polymorph
Lymphocytes
Eosinophils
Globulin
Total Protein Binding

10 METHODS AND MATERIALS:

10.1 Collection and Authentication:

The fruit of *Persea Americana Mill* (Lauraceae) were collected from the local source, Koyambedu Market, Tamil Nadu, Chennai in the month of March.
The plant material was identified and authenticated by Prof P.Jayaraman, Ph.D, Director: Professor, Presidency College, and Chennai 600005. PLANT ANATOMY RESEARCH CENTRE. Tambaram, Tamil Nadu, Chennai-600045. Reg.No PARC/2017/3433. A voucher specimen was submitted at C.L. Baid Metha College of Pharmacy, Chennai-97.

The leaves of *Aloe vera (L.) Burm.f.* (Asphodelaceae) were collected from the local source, Tamil Nadu, Chennai in the month of March.
The plant material was identified and authenticated by Prof P.Jayaraman, Ph.D, Director: Professor, Presidency College, and Chennai 600005. PLANT ANATOMY RESEARCH CENTRE. Tambaram, Tamil Nadu, Chennai-600045. Reg.No PARC/2017/3431. A voucher specimen was submitted at C.L. Baid Metha College of Pharmacy, Chennai-97.

The dried root powder of *Withania somnifera* (Solanaceae) was collected from the local source, Broadway, Tamil Nadu, Chennai in the month of March.
The powder material was identified and authenticated by Prof P.Jayaraman, Ph.D, Director: Professor, Presidency College, and Chennai 600005. PLANT ANATOMY RESEARCH CENTRE. Tambaram, Tamil Nadu, Chennai-600045. Reg.No PARC/2017/3432. A voucher specimen was submitted at C.L. Baid Metha College of Pharmacy, Chennai-97.

10.2 Extraction of ASVERADO:

1. Preparation of Ethanolic extract of Avocado:

The fruit of *Persea Americana Mill* were collected. The raw material such as fruit and seeds were collected separately. Then the fruits were cut into small pieces and it was transferred into a white polyethylene cover. And it was kept in a deep freezer at 2-5ºC for about 5-7 Days. The seeds which were collected separately were shade dried for 5 days.

After the 7 days of freezing, the fruits were blended in a mixer and made into fine powders which weigh about 400g and dried seeds were also blended and made into coarse powder which weighs about 200g.

Pieces of Avocado in Deep Freeze

Freezed Avocado

Fine powder of Avocado

Avocado Seeds

Dried Avocado Seeds

Extraction via Soxhlet Apparatus:

Dried powders of both seeds were transferred into Soxhlets apparatus. Soxhlet apparatus consists of a boiler and reflux which circulates the solvents, a thimble which retains solids to be laved.

The powder to be extracted is introduced into the thimble. The extraction solvent (Ethanol) to be placed used is placed in a distillation flask. The distillation flask is placed on heating mantle. Heating mantle is maintained at a temperature of 40-50◦C.

The solvent is heated, and the vapours move towards distillation arms. The flow of water should be maintained. The non-volatile compounds dissolves in the solvent. At the end of this process, the extract remains in the distillation flask.

After extraction process, if some amount of alcohol remains in the extract it can be evaporated by means of distillation process. In this process, if any traces of alcohol is present in the extract it can be evaporated and collected in a separate receiving flask.

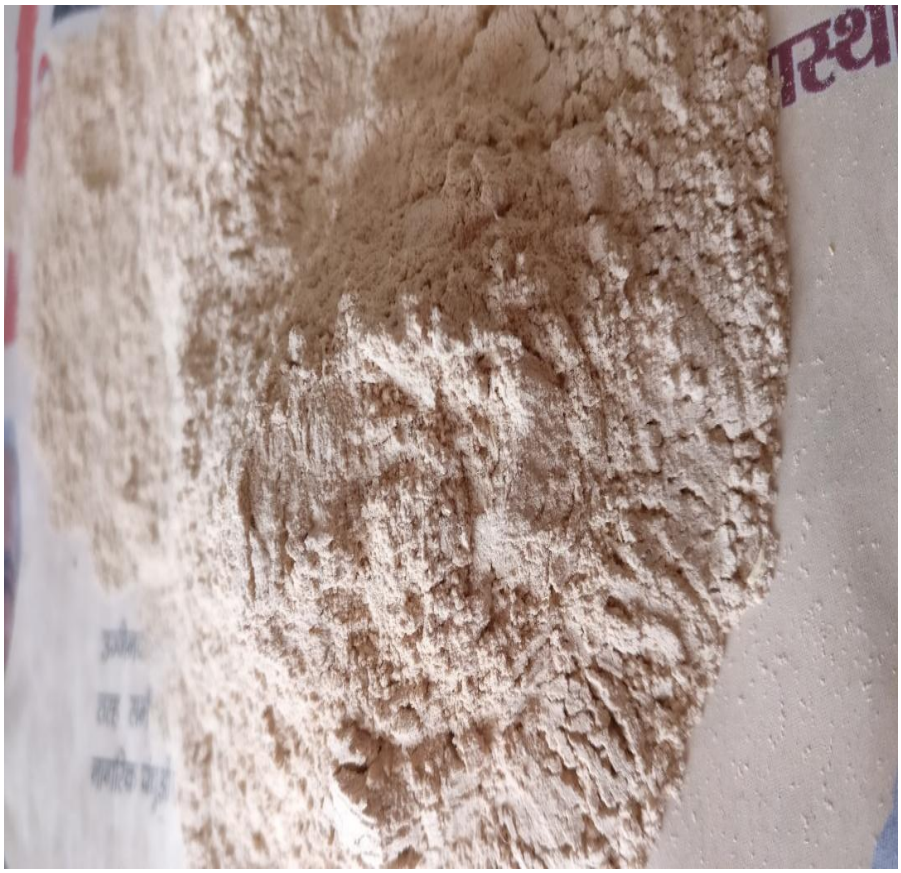
Distillation apparatus consists of round bottom flask, heating mantle, condenser, L-tube, receiving flask, water inlet & outlet.

At the end of this process, the extracted crude compound obtained is a viscous mass.

2. Preparation of Ethanolic extract of Ashwagandha:

The dried root powder of *Withania somnifera* was collected and it weighs about 500g. The powdered roots were extracted with ethanol in Soxhlet apparatus to obtain the viscous mass of crude drug.

Extraction method is same as mentioned above in Avocado preparation



Powdered root of Ashwagandha

3. Extraction of aloe vera juice:

The fresh leaves of *Aloe vera (L.) Burm.f* were collected. The leaves were peeled off and gel was separated. The gel was collected in a vessel. Separated gel was grinded in a blender to obtain a white pulpy juice.

Freshly prepared juice was administered to the experimental animals.

Pieces of Aloe vera gel

Aloe vera juice

10.3 Preparation of ASVERADO:

Crude product obtained from Avocado, Ashwagandha, Aloe vera after the extraction were mixed together in a geometric proportion ratio of 4:4:2 or 2:2:1 (i.e,) ***Avocado (4) : Aloe vera (4) : Ashwagandha (2).***

This proportion ratio is used throughout the experiment for studying Acute toxicity, In-vitro studies & In-vivo studies.

10.4 CHEMICALS REQUIREDS:

All the Chemicals used in the study were of analytical grade.
The following chemicals were used for the experimental study.

Name of the chemicals and their source

SL. no	MATERIALS	SOURCE
1	Chloroform	S.d.fine chemicals Ltd, Mumbai
2	Distilled water	Andavar distilled water
3	DMBA (7,12 Dimethyl benz[a]anthracene)	Sigma -Aldrich, Merck
4	Disodium phosphate	S.d.fine chemicals Ltd, Mumbai
5	Disodium EDTA	Chemspure, Chennai
6	Ether	S.d.fine chemicals Ltd, Mumbai
7	Ethanol	S.d.fine chemicals Ltd, Mumbai
8	Formaline	S.d.fine chemicals Ltd, Mumbai
9	Monosodium phosphate	S.d.fine chemicals Ltd, Mumbai
10	Paclitaxel	Sigma -Aldrich, Merck
11	Sesame oil	Sri Sabari Oil Mills
12	Sodium chloride	Paxy specialities Pvt Ltd
13	Sodium CMC	Scope ingredients Pvt Ltd, Chennai
14	MCF 7 Cell line	National Centre for Cell Sciences, Pune (NCCS)

10.5 PRELIMINARY PHYTOCHEMICAL ANALYSIS[105]:

The ethanolic extract of ASVERADO was subjected to preliminary phytochemical screening for the presence or absence of phyto-constituents by the following methods.

1. Test for alkaloids:

The extract was treated with dilute hydrochloric acid and filtered. The filtrate is used in the following tests.

a) Mayer's reagent (Potassium Mercuric Iodine Solution)

0.5ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid.

b) Dragendroff's test (Potassium Bismuth Iodide)

0.5ml of the extract was treated with Dragen droff's reagent and the appearance of reddish brown color precipitate indicates the presence of alkaloid.

c) Hager's test (Saturated solution of Picric acid)

0.5ml of the extract was treated with Hager's test and the appearance of yellow color precipitate indicates the presence of alkaloid.

d) Wagner's test (Iodine-Potassium Iodide Solution)

0.5ml of the extract was treated with Wagner's test and the appearance of brown color precipitate indicates the presence of alkaloid.

2. Test for Carbohydrates:

a) Molisch's test

The extract was treated with 3ml of alpha-napthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet color ring at the junction of two liquids indicates the presence of carbohydrates.

b) Fehling's test (CuSO4.7H2O+KOH+Potassium Tartarate)

The extract was treated with Fehling's solution A and B heated in boiling water for few minutes. The appearance of reddish brown color precipitate indicates the presence of reducing sugars.

c) Benedict's test (Sodium citrate + sodium carbonate + CuSO4.7H2O)

The extract was treated with Benedict's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of reducing sugars.

d) Barfoed's test (Copper Acetate+ Glacial acetic acid)

The extract was treated with Barfoed's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of non-reducing sugars.

3. Test for steroids

a) Libermann Burchard test:

The extract was treated with small quantity of concentrated sulphuric acid, glacial acetic acid and acetic anhydride. The appearance of green color indicates the presence of steroids.

4. Test for proteins:

a) Biuret's test:

The extract was treated with copper sulphate and sodium hydroxide solution. The appearance of violet color indicates the presence of proteins.

b) Millon's test:

The extract was treated with Millon's reagent. The appearance of pink color indicates the presence of proteins.

5. Test for Tannin's

a) The extract was treated with 10% lead acetate solution. The appearance of white precipitate indicates the presence of tannins.

b) The extract was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

6. Test for Phenols

a) The extract was treated with neutral ferric chloride solution. The appearance of violet indicates the presence of phenols.

b) The extract was treated with 10% sodium chloride solution. The appearance of cream color indicates the presence of phenols.

7. Test for Flavonoid's

a) 5ml of extract solution was hydrolysed with 10%v/v sulphuric acid and cooled. Then, it is extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

b) **Shinoda's test:** The extracts were dissolved in alcohol, to that one piece of magnesium is added followed by concentrated hydrochloric acid along the sides of the test tube drop wise. It is heated in a boiling water bath for few minutes. The appearance of magenta colour indicates the presence of flavonoids.

8) Test for Gums and Mucilage

The extract was treated with 25ml of absolute alcohol and then solution was filtered. The filtrate was examined for its swelling properties.

9) Test for Glycosides

The extract was dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides.

10) Test for Saponins

1ml of the extract was diluted to 20ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

11) Test for Terpenes

The extract was treated with tin and thionyl chloride, appearance of pink color indicates the presence of terpenes.

12) Test for sterols

The extract was treated with 5% potassium hydroxide solution; appearance of pink color indicates the presence of sterols.

11. In vitro screening[99]:

There are many in vitro methods available for testing anti cancerous activity of a compound and the primary evaluation technique is cell culture. *In vitro* methods are less expensive as well as less time-consuming compared to *in vivo* tumor models, thereby allowing evaluation of large quantities of new anticancer agents. Sophisticated *in vitro* experiments provide data on mechanisms of action, by how the compounds act on tumour entities when combined with the specific cell lines. Based on these data, further selection of promising drugs for *in vivo* testing can be obtained.

In vitro methods[99]:

There are different types of assays which are used in the in-vitro studies of anti-cancer. They are

- Tetrazolium salt assay (MTT or Microculture Tetrazolium Test)
- Sulphorhodamine B assay
- ^{3}H-Thymidine Uptake assay
- Dye Exclusion Test
- Clonogenic assay
- Cell counting assay

Procedure:

Cell line and culture[104]:

MCF 7 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C.

In Vitro assay for anti cancer activity: (MTT assay)

Cells (1 × 105 /well) were plated in 24-well plates and incubated in 370C with 5% CO2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells .The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells / A570 of control cells × 100

12.1 ACUTE TOXICITY STUDIES[106]:

The procedure was followed by using OECD 423 guidelines 423.The acute toxic class method (423) is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or morbidity status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the substances. This procedure results in the use of a specified number of animals while allowing for acceptable data- based scientific conclusion.

The method used defined doses (2000mg/kg body weight) and results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for classification of chemical which cause acute toxicity.

PROCEDURE:

Adult female Wistar rats, weighs about 180-250kg were used for the study. The dose level of Alcoholic extract of ASVERADO was 2000mg/kg body weight p.o. as most of the crude extract posses LD50 value more than 2000mg/kg, p.o. so starting dose used was 2000mg/g p.o. Dose volume administered was 4ml/100 gm body weight to rats which were fasted overnight with water *ad libitum*. Food was withheld for further 3-4hrs after oral administration of drugs. The animals are observed individually after dosing at least once during first 30 minutes than periodically during the first 24 hours with special attention given during the first 4 hours and thereafter for 14 days for any toxic signs and symptoms.

EVALUATION:

Body weight of mice's before and after determination were noted and any changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic & central nervous system, motor activity and behavior pattern were observed and also sign of tremors, convulsion, salivation, diarrhoea, circling,

depression, excitement, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted.

12.2 EXPERIMENTAL DESIGN:

Female Wistar rats at age of 50 days and above were obtained from the animal house of C.L.Baid Metha College of Pharmacy , Thoraipakkam, Chennai- 97, and housed in groups of six at 22±2◦C in acrylic cages (26cm×48cm×21 cm) for 7 days for acclimation to the animal colony, with 12hrs light/dark cycle and access to protein diet which consists of various ingredients such as *47% Chick pea, 13% Wheat flour, 10% Red kidney bean, 7% Soy beans, 5% Ground nut, 3% Milk powder, 10% Split Red Lentil,5% Melon seeds* and glucose water ad libitum. Post-surgical housing was individual for 7 days to allow recovery. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of

CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

IAEC NO: IAEC/L/05/CLBMCP/2017

After recovery of rats, the animals were assigned as follows:

SL.no	GROUPING	TREATMENT	DURATION
1	Group-I Control	Saline water, p.o	13 Weeks
2	Group-II Negative control	DMBA in sesame oil, p.o / i.p	13 Weeks (Two doses) 1st week & 5th week
3	Group-III Standard	Paclitaxel in saline, i.p	From 9th week- 13th Week(Weekly dosing)
4	Group-IV Low Dose (mg)	ASVERADO in sodium CMC, p.o	From 9th week- 13th week(Regular dosing)
5	Group-V High Dose (mg)	ASVERADO in sodium CMC, p.o	From 9th week- 13th week(Regular dosing)

12.3 INDUCTION OF DMBA, ASVERADO & PACLITAXEL:

DMBA

- By Intragastrically (1st week)

DMBA was given intragastrically by gavage using cannula fitted to a feeding needle.

- In flank region (5th week)

DMBA was given intraperitoneally in the flank region.

To prepare a dose of 20mg/ml of DMBA, 20mg of DMBA powder was dissolved in the 1ml of sesame oil. Standard dose of DMBA is 20mg/ml

ASVERADO

The extract was given intragastrically by gavage using cannula fitted to a feeding needle.

To prepare an ASVERADO extract solution, the dose is calculated based on the animal weight and dissolved in the 0.5% Sodium CMC. Dose is calculated using below mentioned formula:

$$\text{Dose administered} = \frac{\text{Body weight} \times \text{Standard dose}}{1000}$$

Extract high dose is 400mg/kg*

Extract low dose is 200mg/kg*

NOTE*: The high dose and low dose value are selected based on the acute toxicity studies.

PACLITAXEL

The standard drug Paclitaxel was given intra peritoneally in normal saline. Standard dose of paclitaxel is 1mg/kg.

To prepare a Paclitaxel solution, the dose is calculated based on animal weight and dissolved in 0.9% saline.

METHODOLOGY:

GROUP I (Normal control group): Animals were treated with only saline water for 13 weeks.

GROUP II (DMBA administered control group): Animals were subjected to a two doses of 20mg of DMBA in sesame oil.

GROUP III (Standard group): Animals were treated with 20mg/ml of two doses of DMBA + 1mg/ml/week of Paclitaxel in Saline solution.

GROUP IV (Extract Low dose): Animals were treated with 20mg/ml of two doses of DMBA + 200mg/ml/day of extract.

GROUP V: (Extract High dose): Animals were treated with 20mg/ml of two doses of DMBA + 400mg/ml/day of extract.

12.4 TERMINATION OF EXPERIMENTAL ANIMALS:

After the 13 weeks of experimental duration, the blood was collected from the animals by retro-orbital puncture & by cervical dislocation for evaluation of Blood parameters and Anti-oxidant assay.

Immediately after the collection, the blood was centrifuged for 10 min at 3000rpm. After the centrifugation, the serum and plasma gets separated. The plasma is discarded and serum is evaluated for the Serology study such as RBC, WBC, Blood sugar level, Hb, Ht, total protein binding,SOD, ESR,Eisnophils.

Breast,Liver and Kidney were dissected from the body and transferred to a container containing 10% Buffered Formalin Solution for the evaluation of histopathological studies.

PARAMETERES:

Effect of haematological parameters:

The effect on haematological parameters in rats following 13 weeks oral administration of ethanolic extracts of *ASVERADO* for toxicity study are presented. Percent haemoglobin (% Hb) and total erythrocyte count (TEC) were found to decretase significantly ($p<0.05$) in ASVERADO treated animals compared to control group animals. Packed cell volume (PCV) also decreased in the ASVERADO group but the decrease was non-significant.

There was no change in total leucocyte count (TLC) in TPHE treated animals although differential leucocyte count chamged significantly ($p<0.05$) in case of lymphocytes, monocytes, neutrophils and eosinophils. Neutrophils and eosinophils were found to increase in ASVERADO treated animals while lymphocytes and monocytes were decreased compared to control.

Serum biochemical profile

The effect of ASVERADO on serum biochemical parameters in mammary tumour bearing rats following 13 weeks oral administration The levels of total protein, albumin and globulin were found to decrease significantly ($p<0.05$) in animals of cancer control group compared to normal animals while these values increased significantly in all the treatment groups compared to cancer control group.

Oxidative stress related parameters:

Estimation Of Antioxidant Enzymes:

Reagents

1. Carbonate buffer (100mM, pH 10.2)

2. Epinephrine (3mM)

Procedure

The SOD activity in supernatant was measured by the method of Misra and Fridovich. The supernatant (500µl) was added to 0.800ml of carbonate buffer (100mM, pH 10.2) and 100µl of epinephrine (3mM). The change in

absorbance of each sample was then recorded at 480nm in spectrophotometer for 2min at an interval of 15sec. Parallel blank and standard were run for determination of SOD activity.

One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation.

Reagents	**Uninhibited (Standard)**	**Inhibited (Sample)**	**Blank**
Carbonate buffer	0.900ml	0.800ml	1.0ml
Supernatant	-	0.1ml	-
Epinephrine	0.1ml	0.1ml	-

The reaction mixtures are diluted 1/10 just before taking the readings in spectrophotometer

Calculation

$$\% = \frac{\Delta A_{480nm}/\text{min Uninhibited} - \Delta A480nm/\text{min inhibited}}{{}^{\Delta A}480nm/\text{min Uninhibited} - \Delta A_{480nm}/\text{min Blank}} \times 100$$

$$/ = \frac{\%\text{Inhibition} \times V_t}{(50\%) \times V_s}$$

$$/ = \frac{\text{Units / ml enzyme}}{\text{mg protein / ml enzyme}}$$

Where,

V_t = Total volume (1.0ml)

Estimation of Catalase (CAT)

Reagents

1.Phosphate buffer solution (50mM)

i. Dissolve 6.81g of KH_2PO_4 in 1000ml distilled water

ii. Dissolve 6.9g of Na_2HPO_4 in 1000ml distilled water

390ml from solution (A) are mixed with 610ml from solution (B), the pH is adjusted to 7

2.Hydrogen peroxide (H_2O_2) 30mM

0.34ml of 30% H_2O_2 is diluted with phosphate buffer to 100ml

Procedure

Catalase activity was measured by the method of Aebi. 0.1ml of supernatant was added to cuvette containing 1.9ml of 50mM phosphate buffer (pH

7.0). Reaction was started by the addition of 1.0ml of freshly prepared 30mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240nm. Activity of catalase was expressed as units/mg protein. A unit is defined as the velocity constant per second.

Reagents	**Sample**	**Blank**
Phosphate buffer	1.9ml	2.9ml
Supernatant	0.1ml	0.1ml
H2O2	1 ml	-

The reaction occurs immediately after the addition of H_2O_2.

Solutions are mixed well and the first absorbance (A_1) is read after 15sec (t_1) and the second absorbance (A_1) after 30sec (t_1). The absorbance is read at wavelength 240nm.

Calculation

$$\mathbf{K} = Vt/Vs \times 2.3/\Delta t \times \log A1/A2 \times 60$$

Where,

K =Rate constant of the reaction

$\Delta t = (t2-t1) = 15sec$

A1 = Absorbance after 15sec

A2 = Absorbance after 30sec

Vt = Total volume (3ml)

Vs = Volume of the sample (0.1ml)

Estimation of Lipid peroxidase (LPO)

The level of Lipid peroxidase was estimated by Thiobarbituric acid reaction method described by Ohkawa *et al*

Reagents

1. Sodium dodecyl sulphate (SDS) (8.1% w/v)

2. Acetic acid (20%; pH 3.5)

3. Thiobarbituric acid (TBA) (0.8%)

4. n-butanol/pyridine mixture (15:1)

Procedure

To 0.2ml of test sample, 0.2ml of SDS, 1.5ml of acetic acid and 1.5ml of TBA were added. The mixture was made up to 4ml with water and then heated in a water bath at 95°C for 60min. After cooling, 1ml of water and 5ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000rpm for 10min, the organic layer was taken and its absorbance was read at 532nm. The level of lipid peroxides was expressed as nmoles of MDA released/g wet tissue.

Reagents	Sample	Blank
SDS	0.2ml	0.2ml
Supernatant	0.2ml	-
DDW	1.6ml	108ml
Acetic acid	1.5ml	1.5ml
TBA	1.5ml	1.5ml
n-butanol/pyridine	5ml	5ml

Calculation:

Concentration of MDA= Absorbance at 532nmL $\times \epsilon \times D$

Where,

L = Light path (1cm)

ϵ = Extinction co-efficient 1.56x105 M-1cm-1

$$D = \frac{\text{Total volume (10ml)}}{\text{Volume of the sample (0.2ml)}}$$

Methods for Histopathological study:

The rats from each group were anaesthetized using intraperitoneal injection of sodium pentobarbital (50mg/kg). The organs such as Breast, Kidney, Liver, Spleen was carefully removed without injury. The collected organs were washed with ice cold normal saline and fixed in 10% formal saline (10ml of formaldehyde in 90ml of physiological saline dye. The sections were examined microscopically for histopathological changes.

Statistical Analysis:

The statistical analysis was carried by one way ANOVA followed by Dunnet's "t" test. P value <0.05 (95% confidence limit) was considered statistically significant, using software Graph Pad Prism version6

RESULTS:

Extraction yield:

The alcoholic of extract of Ashwagandha was dark chocolate brown in colour and was stored in refrigerator until use. The percentage yield of the extract was ***13.02% w/w.***

The alcoholic extract of Avocado was dark chocolate brown in colour and was stored in refrigerator untilcent use. The percentage yield of the extract was ***16.22%w/w***

The fresh juice of aloe vera was white pulpy in colour. The percentage yield was ***5%w/v***

Preliminary Phytochemical study:
The ethanolic extract of ASVERADO showed presence of various Phyto-chemical constituents such as alkaloids, carbohydrate, proteins, tannins, steroids, glycosides, flavonoids, and saponins. Results are shown in **Table 1**

In-vitro assay for Anti-cancer activity (Cell line):

The ethanolic extract of ASVERADO showed effect on ***MCF 7*** cell line with the 49.43% of inhibition of cell viability at 125µg/ml of concentration. Results are shown in **Table 2, Graph 1**

Acute Toxicity study:

According to the OECD guideline 423 (Acute toxic class method) acute oral toxicity studies were performed. A single starting dose of 2000mg/kg p.o was administered to the animal and observed for three days. There was no significant change in body weight of the animals before and after treatment of drug and no signs of toxicity was found and the animals were observed for 14 days. No signs of toxicity were found. Results are shown in **Table 3**.

EFFECT OF HAEMOTOLOGICAL PARAMETER:

The Group II animals showed a significant decrease in Haematological parameter when compared to the Group I animals. Treatment with AVERADO (200&400 mg/kg) significantly increased the Haematological blood parameter (Group III & Group IV) on comparison with Group II. There was significant difference in the repetitive / stereotypic behavior of both Group I and Group II animals. Treatment with ASVERADO (200& 400mg/kg) significantly decreased the number and duration of repetitive / stereotypic

activity. Results are given in **Table4 & Table 5** and represent, **Graph 2, Graph 3, Graph 4, Graph 5, Graph 6**

Serum biochemical profile:

The effect of ASVERADO on serum biochemical parameters in mammary tumour bearing rats following 13 weeks oral administration The levels of total protein, albumin and globulin were found to decrease significantly ($p<0.05$) in animals of cancer control group compared to normal animals while these values increased significantly in all the treatment groups compared to cancer control group. This recovery was maximum in ASVERADO 400 when compared with ASVERADO 200. Results are given in **Table** 6 and represents**, Graph 7, Graph 8**

Estimation of Antioxidant Enzymes:

Effect of ASVERADO on Superoxide dismutase:

SOD level in the Breast of Group II animals was decreased significantly on comparison with Group I animals. Treatment with AVERADO 200mg/kg and 400mg/kg (group III & Group IV) showed significant increase in SOD level on comparison with Group II animals. Results are given in Table 7 and represents, Graph 9

SOD level in the Kidney of Group II animals was decreased significantly on comparison with Group I animals. Treatment with AVERADO 200mg/kg and 400mg/kg (group III & Group IV) showed significant increase in SOD level on comparison with Group II animals. Results are given in Table 7 and represents, Graph 9

SOD level in the Liver of Group II animals was decreased significantly on comparison with Group I animals. Treatment with AVERADO 200mg/kg and 400mg/kg (group III & Group IV) showed significant increase in SOD level on comparison with Group II animals. Results are given in Table 7 and represents, Graph 9

SOD level in the Spleen of Group II animals was decreased significantly on comparison with Group I animals. Treatment with AVERADO 200mg/kg and 400mg/kg (group III & Group IV) showed significant increase in SOD level on comparison with Group II animals. Results are given in Table 7 and represents, Graph 9

Effect of ASVERADO on Catalase:

Catalase level in the Breast of Group II animals were found decreased significantly on comparison with Group I animals. Treatment with ASVERADO 200

and 400mg/kg (Group III& Group IV) showed significant increase in catalase level on comparison with Group II animals. Table 8 and represents, Graph 10

Catalase level in the Kidney of Group II animals were found decreased significantly on comparison with Group I animals. Treatment with ASVERADO 200 and 400mg/kg (Group III& Group IV) showed significant increase in catalase level on comparison with Group II animals. Table 8 and represents, Graph 10

Catalase level in the Liver of Group II animals were found decreased significantly on comparison with Group I animals. Treatment with ASVERADO 200 and 400mg/kg (Group III& Group IV) showed significant increase in catalase level on comparison with Group II animals. Table 8 and represents, Graph 10

Catalase level in the Spleen of Group II animals were found decreased significantly on comparison with Group I animals. Treatment with ASVERADO 200 and 400mg/kg (Group III& Group IV) showed significant increase in catalase level on comparison with Group II animals. Table 8 and represents, Graph 10

Effect of ASVERADO on Lipid peroxidation

Lipid peroxidation in the Breast of Group II animal were found increased significantly on comparison with ASVERADO 200& 400mg/kg (Group III & Group IV) showed significant decrease in lipid peroxidation on comparison with Group II animals. Table 9 and represents, Graph 11

Lipid peroxidation in the Kidney of Group II animal were found increased significantly on comparison with ASVERADO 200& 400mg/kg (Group III & Group IV) showed significant decrease in lipid peroxidation on comparison with Group II animals. Table 9 and represents, Graph 11

Lipid peroxidation in the Liver of Group II animal were found increased significantly on comparison with ASVERADO 200& 400mg/kg (Group III & Group IV) showed significant decrease in lipid peroxidation on comparison with Group II animals. Table 9 and represents, Graph 11

Lipid peroxidation in the Spleen of Group II animal were found increased significantly on comparison with ASVERADO 200& 400mg/kg (Group III & Group IV) showed significant decrease in lipid peroxidation on comparison with Group II animal. Table 9 and represents, Graph 11

Tables & Graphs

Table 1: Preliminary Phytochemical study

SL.no	Constituents	Remarks
1	Alkaloids	Present
2	Carbohydrates	Present
3	Proteins	Present
4	Steroids	Present
5	Phenols	Absent
6	Tannins	Present
7	Glycosides	Present
8	Flavonoids	Present
9	Saponins	Present
10	Terpens	Absent
11	Gum & Mucilage	Absent
12	Sterols	Absent

In-vitro assay for Anti-cancer activity (Cell line):

Table 2: Anticancer effect of Sample *on MCF 7* cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.204	28.57
2	500	1:1	0.255	35.71
3	250	1:2	0.301	42.15
4	125	1:4	0.353	49.43
5	62.5	1:8	0.405	56.72
6	31.2	1:16	0.458	64.14
7	15.6	1:32	0.512	71.70
8	7.8	1:64	0.559	78.29
9	Cell control	-	0.714	100

Calculation:

% Cell viability = A570 of treated cells / A570 of control cells × 100

=0.559/0.714 X 100

=0.7829 X 100

=78.29%

Therefore,

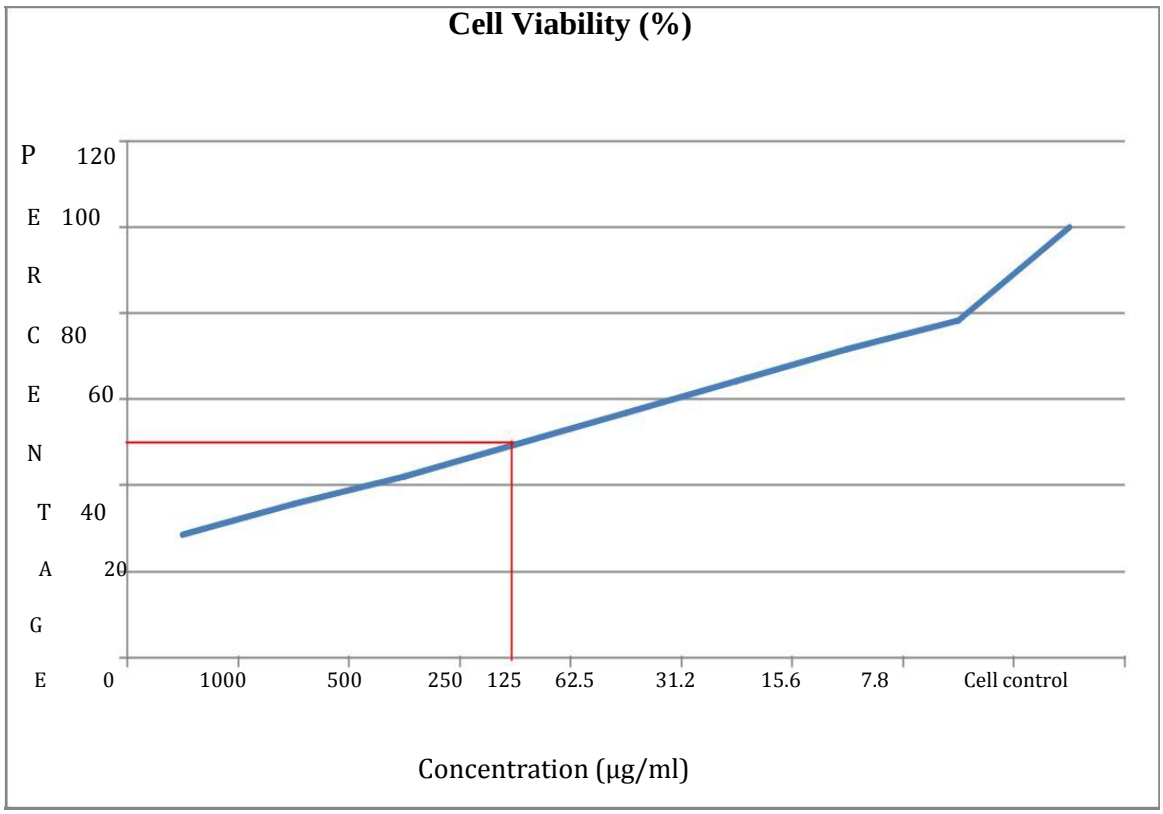
% Cell viability = A570 of treated cells / A570 of control cells × 100

=0.353/0.714X100

=0.4943

% Cell viability =49.43% in 125µg/ml

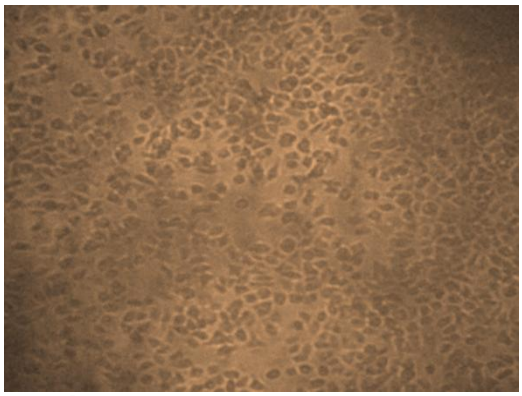
Graph 1: **Anticancer effect of Sample *on MCF 7* cell line**



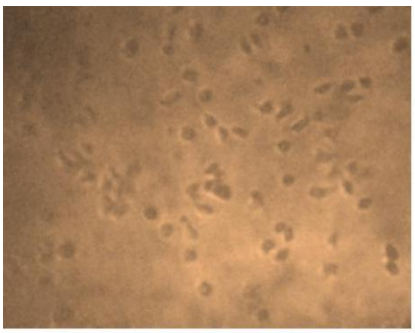
Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

Anticancer effect of Sample on *MCF 7 cell* line

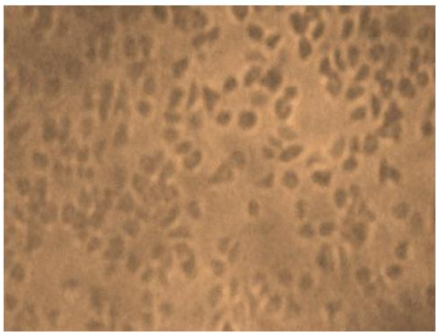
Normal MCF 7 cell line



Toxicity-1000 µg/ml



Toxicity-125 µg/ml



Toxicity- 7.8 µg/ml

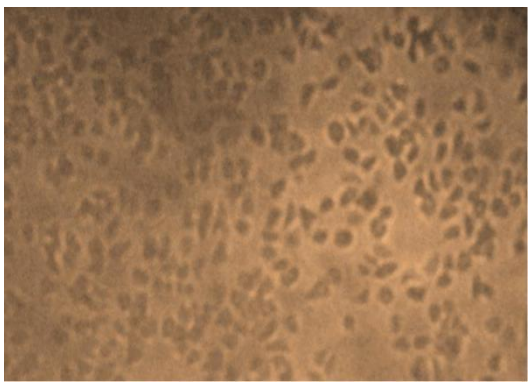


Table 3: Acute oral toxicity results of ASVERADO

SL.no	Treatment	Dose	Weight of animals		Sign of toxicity	Onset of toxicity	Reversible or Irreversible	Duration
			Before (kg)	After(kg)				
1	ASVERADO	2000mg /kg	160	161				14 Days
2	ASVERADO	2000mg /kg	150	150				14 Days
3	ASVERADO	2000mg /kg	160	162	No sign of toxicity	Nil	Nil	14 Days

Evaluation of Blood parameters:

Table 4 & 5: Heamatological Profile of DMBA Induced Mammary gland Cancer:

Table 4

SL.NO	Treatment	Hb (g/dl)	PCV	TEC	TLC
1	Control	12.00 ± 0.43	38.33 ± 1.58	6.38 ± 0.21	9.10 ± 0.37
2	Negative Control	6.78 ± 0.23*	25.60 ± 1.10**	3.47 ± 0.23**	5.34 ± 0.24**
3	Standard	11.95 ± 0.44	35.40 ± 1.20	6.50 ± 0.43	8.16 ± 0.67
4	ASVERADO 200	8.66 ± 0.32	30.78 ± 1.25**	5.53 ± 0.24	7.10 ± 0.46*
5	ASVERADO 400	11.56 ± 0.41*	35.03 ± 1.50	6.28 ± 0.49**	8.88 ± 0.76

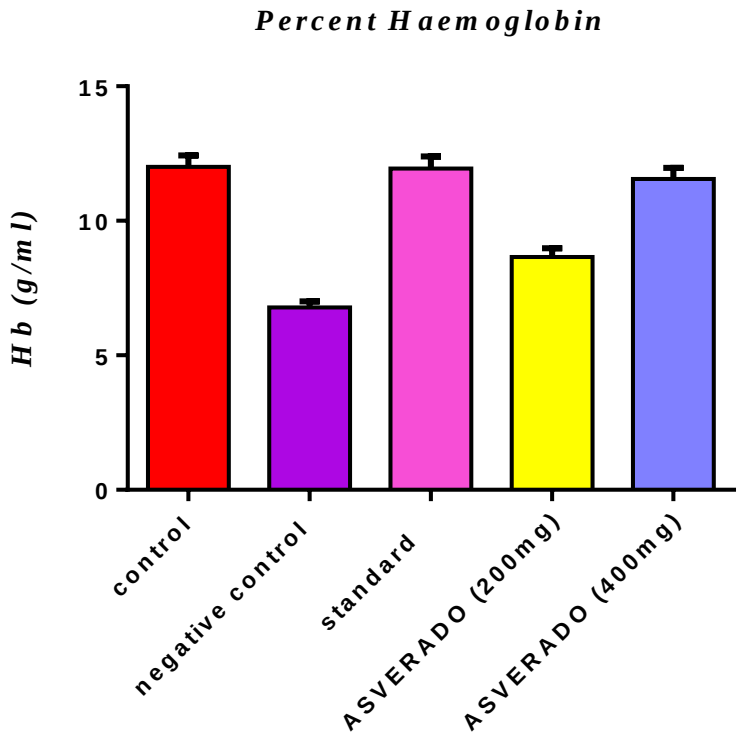
Table 5

SL.NO	Treatment	Lymphocyte	Monocyte	Eosinophil	Basophil
1	Control	72.24 ± 1.67	3.06 ± 0.12	2.12± 0.06**	0.15 ± 0.02
2	Negative Control	35.27± 1.50**	2.54 ± 0.13**	3.17 ± 0.16	0.11 ± 0.11
3	Standard	70.72 ± 2.22	3.01 ± 0.10*	2.57 ± 0.08	0.13 ± 0.01*
4	ASVERADO 200	63.95 ± 1.83*	2.68± 0.10	2.25 ± 0.08*	0.13 ± 0.10
5	ASVERADO 400	68.27 ± 2.10	2.77 ± 0.07	2.47 ± 0.05	0.15 ± 0.01**

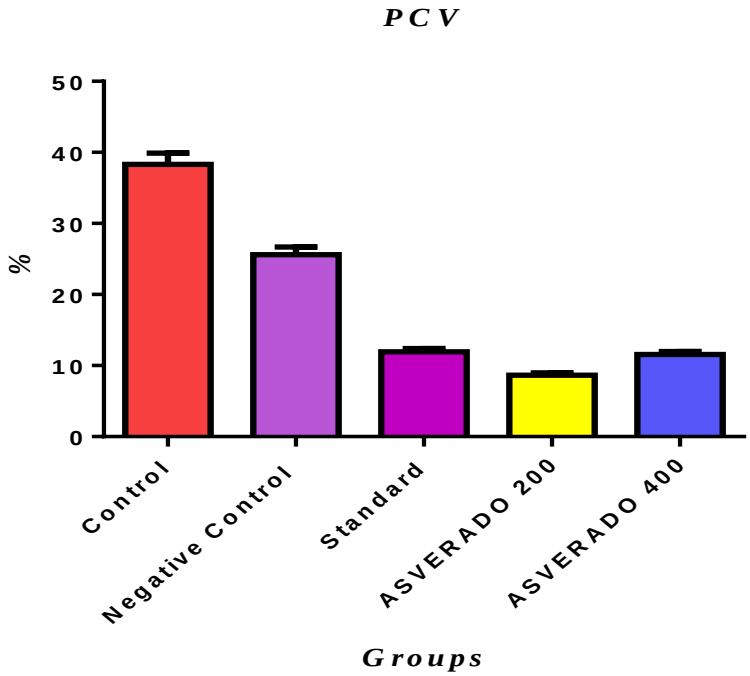
***** Denotes significant difference at $p<0.05$ compared to normal control; *****Denotes significant difference at $p<0.05$ compared to cancer control within column (Mean ± SEM, n=6)

Values are represented in Mean ± SEM, n=6
Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV
Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test
ns- Non significant, *p<0.05, **p<0.01, *p<0.001

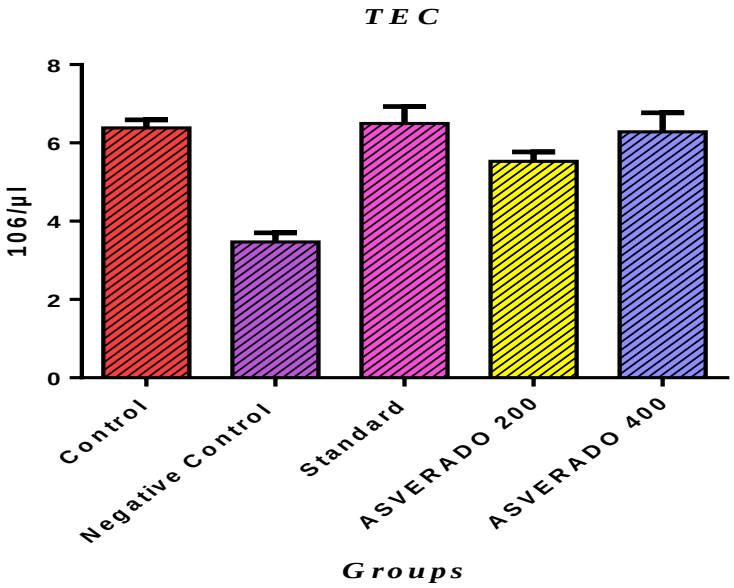
Graph 2 (Haemoglobin count)



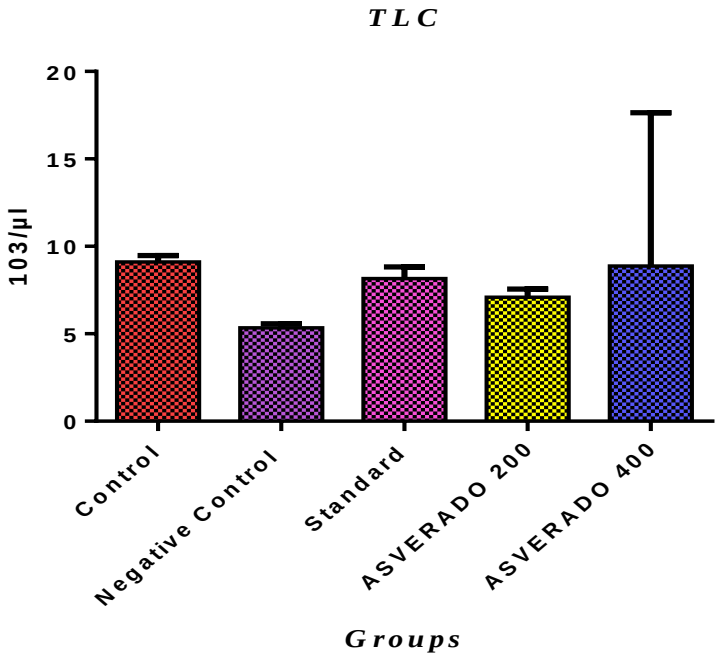
Graph 3 (PCV count)



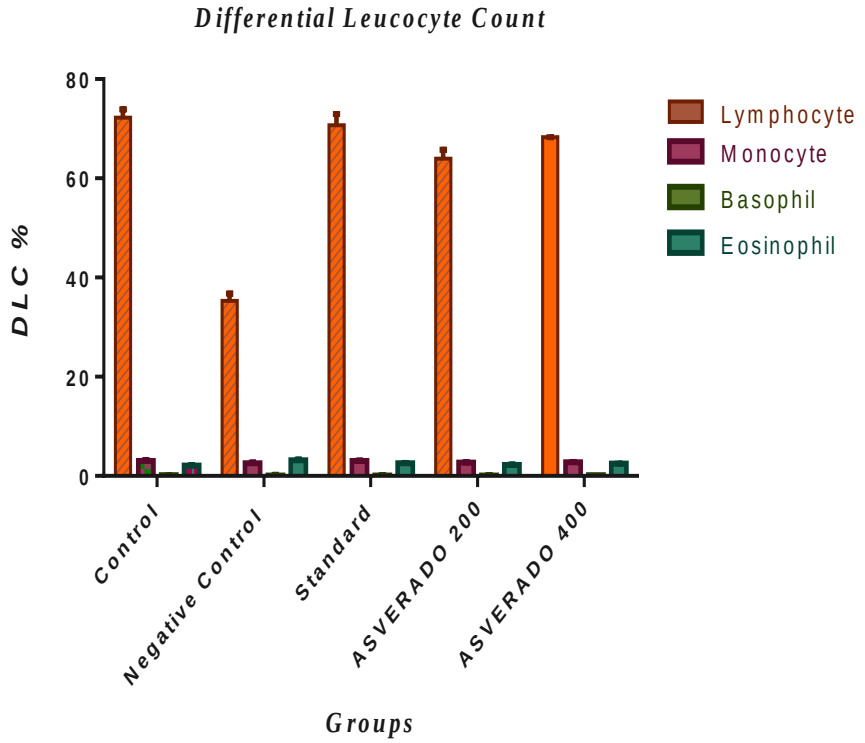
Graph 4 (TEC count)



Graph 5 (TLC count)



Graph 6 (Differential leukocyte count)



EFEECT OF ASVERADO ON SERUM BIOCHEMICAL PROFILE:

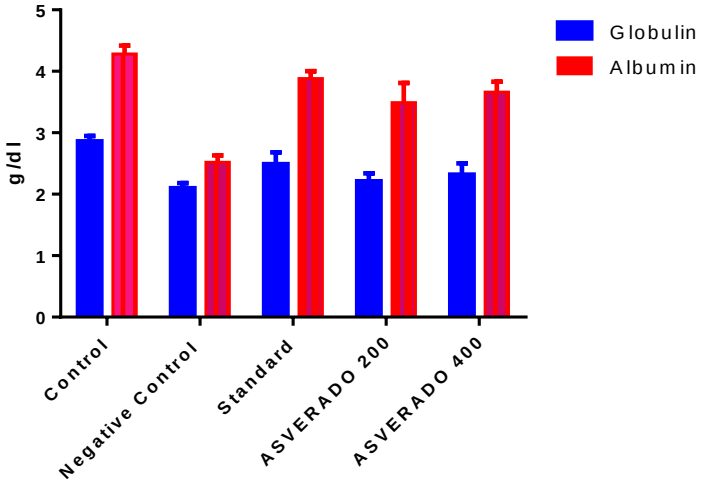
- **TABLE 6: EFEECT OF ASVERADO ON SERUM BIOCHEMICAL PROFILE:**

SL.NO	Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G
1	Control	7.13 ±0.19	4.28 ±0.14	2.86 ±0.09	1.49 ±0.08
2	Negative Control	4.63 ±0.25	2.52 ±0.11*	2.10 ±0.08**	1.21 ±0.07
3	Standard	6.38 ±0.29	3.88 ±0.12*	2.49±0.19**	1.55 ±0.14
4	ASVERADO 200	5.90 ±0.42*	3.49 ±0.32*	2.21 ± 0.13	1.45±0.02
5	ASVERADO 400	6.15 ±0.31*	3.66 ± 0.17	2.32±0.18	1.52±0.10

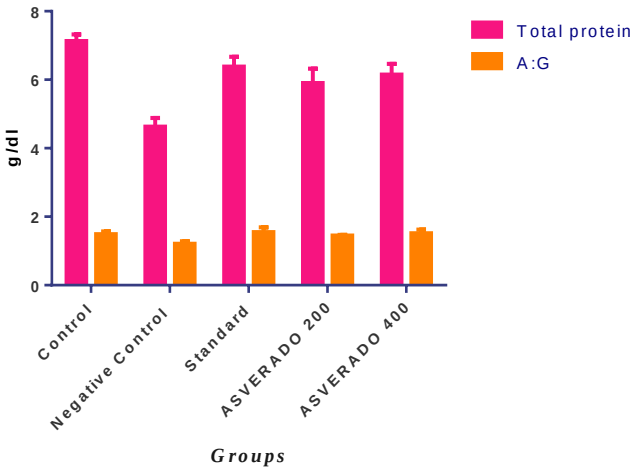
* Denotes significant difference at p<0.05 compared to normal control; *Denotes significant difference at p<0.05 compared to cancer control within column (Mean ± SEM, n=6)

Values are represented in Mean ± SEM, n=6
Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV
Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test
ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph: 7 EFEECT OF ASVERADO ON SERUM BIOCHEMICAL PROFILE:



Graph: 8 EFEECT OF ASVERADO ON SERUM BIOCHEMICAL PROFILE



Evaluation of Anti-oxidant property:

Effect of ASVERADO on Superoxide dismutase

Table 7: Effect of ASVERADO on Superoxide dismutase

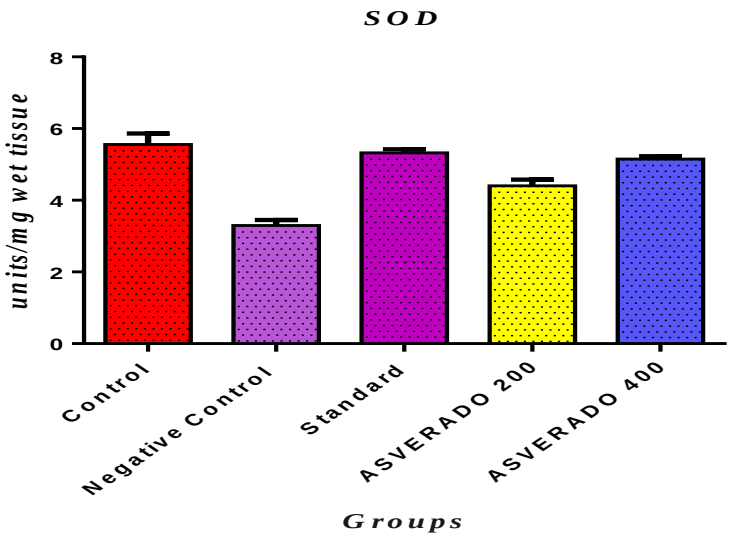
SL.NO	Treatment	SOD (mg/wet tissue)
1	Control	5.56±0.30
2	Negative Control	3.30±0.15***
3	Standard	5.32±0.10
4	ASVERADO 200	4.40±0.18
5	ASVERADO 400	5.15±0.08

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV
Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test

ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 9: EFEECT OF ASVERADO ON SERUM BIOCHEMICAL PROFILE



Effect of ASVERADO on Catalase

Table 8: Effect of ASVERADO on Catalase

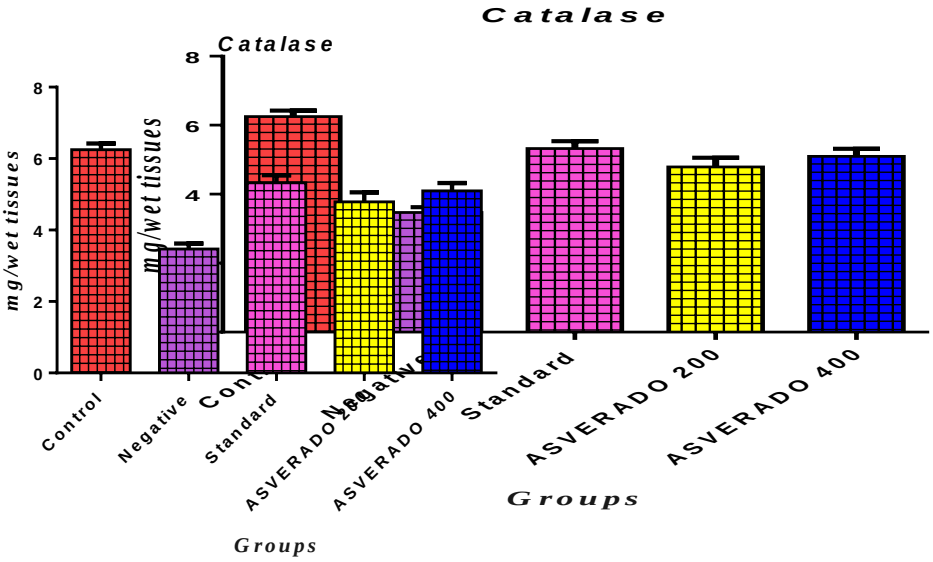
SL.NO	Treatment	Catalase (mg/wet tissue)
1	Control	6.25±0.17
2	Negative Control	3.47±0.15***
3	Standard	5.32±0.22
4	ASVERADO 200	4.79±0.27***
5	ASVERADO 400	5.10±0.22***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV
Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test

ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 10: Effect of ASVERADO on Catalase



Effect of ASVERADO on LIPID PEROXIDASE:

Table 9: Effect of ASVERADO on Catalase:

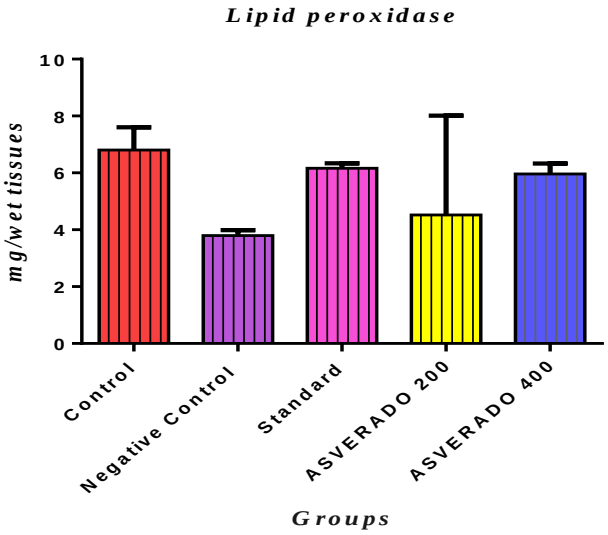
SL.NO	Treatment	Lipid peroxide (mg/wet tissue)
1	Control	6.8±0.38
2	Negative Control	3.88±0.19
3	Standard	6.16±0.18
4	ASVERADO 200	4.52±0.17
5	ASVERADO 400	5.96±0.37

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV
Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test

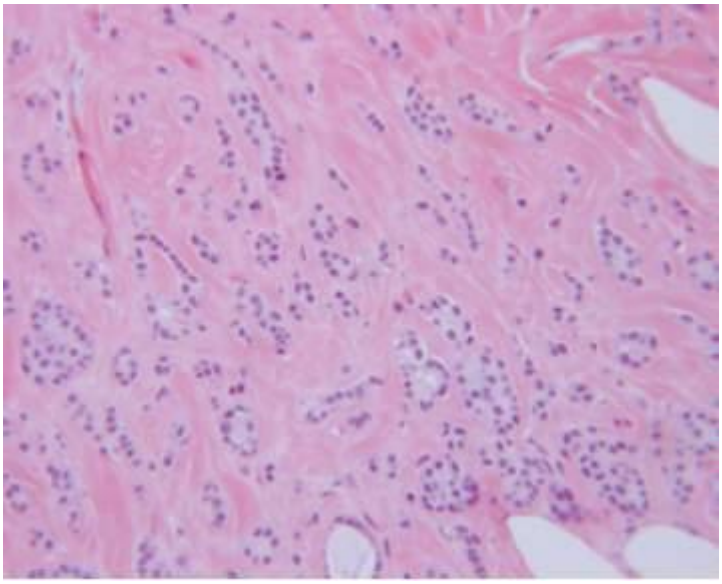
ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 11: Effect of ASVERADO on LIPID PEROXIDASE

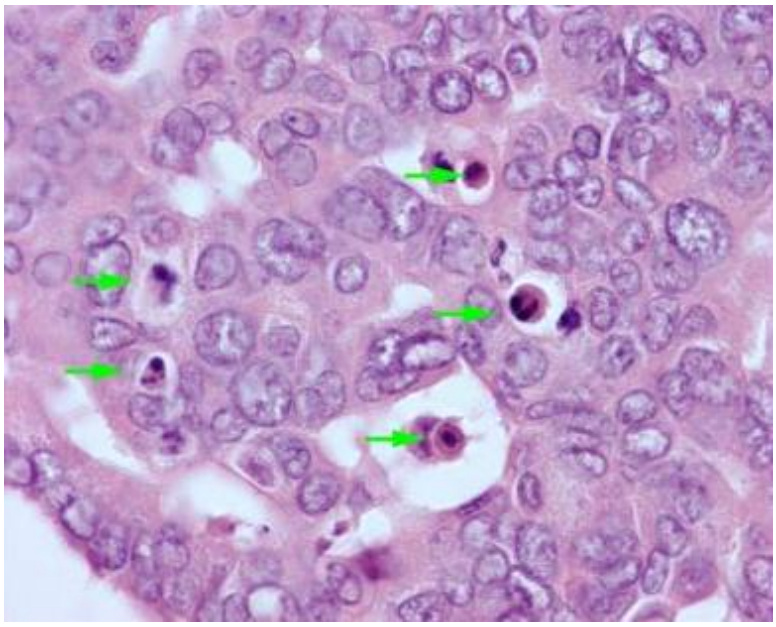


HISTOPHATHOLOGY OF BREAST

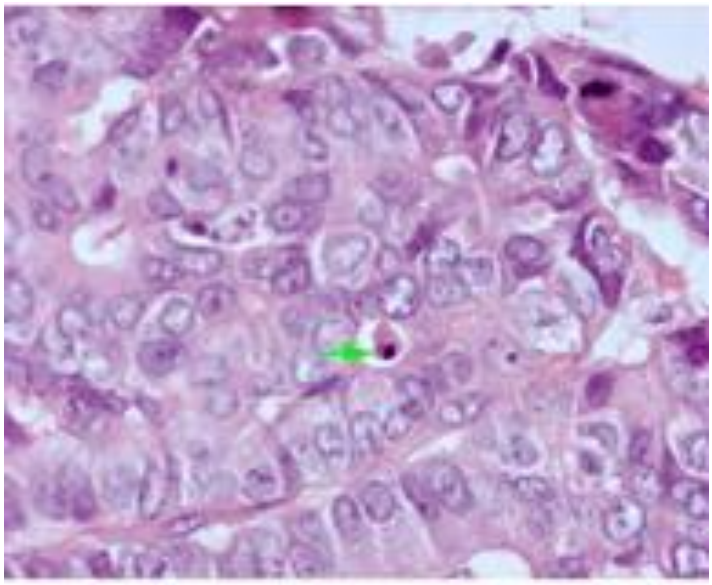
Control Group



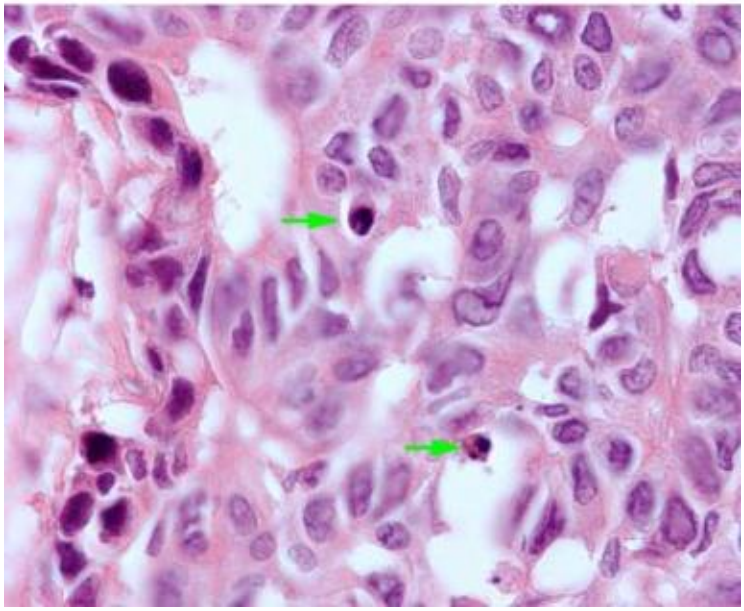
Negative Group



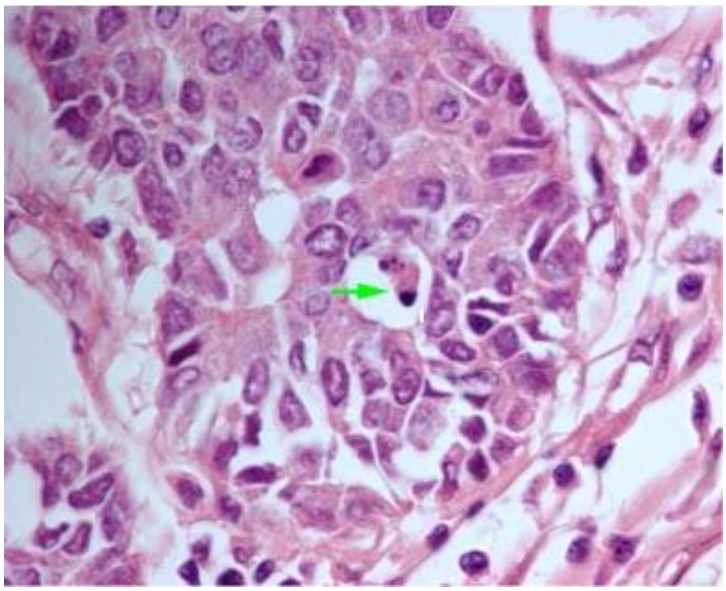
Standard



Low Dose

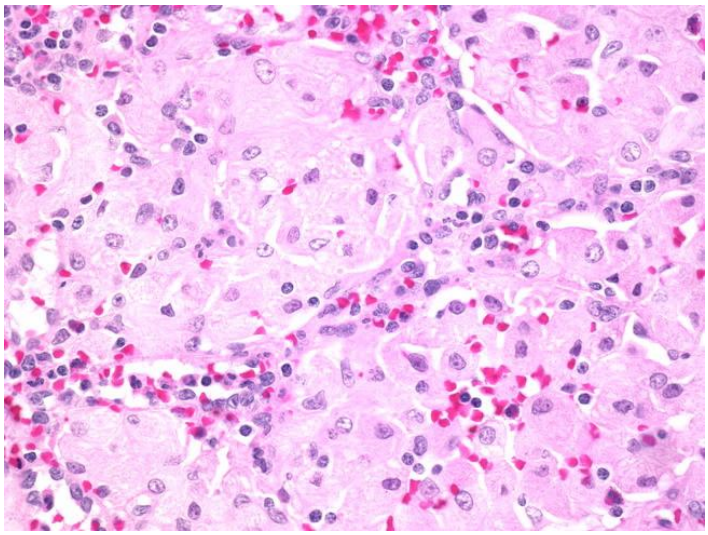


High dose

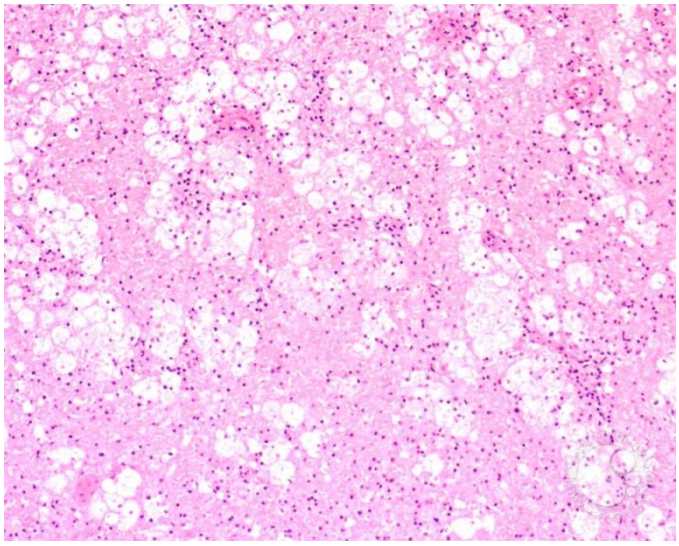


HISTOPATHOLOGY OF SPLEEN:

Control Group

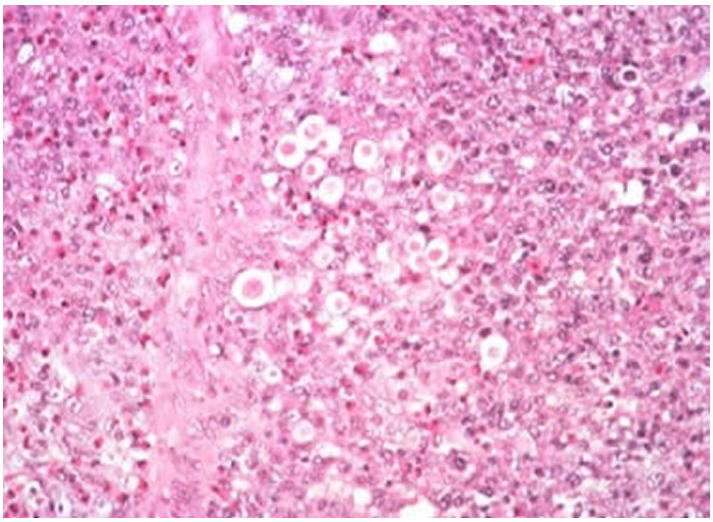


Negative control



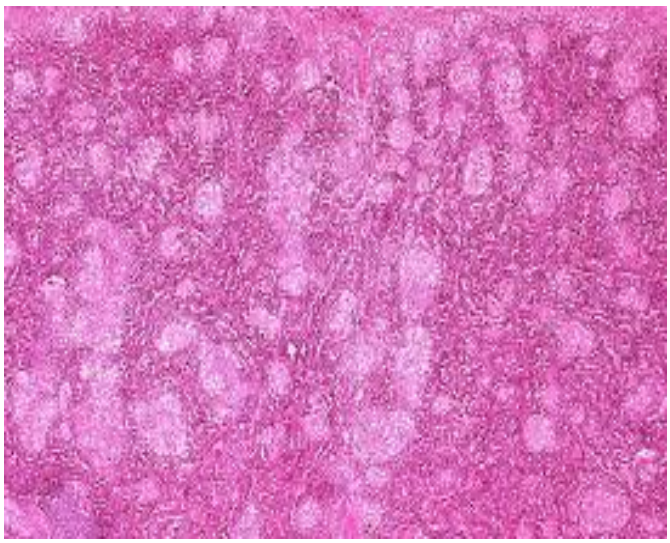
NO TUMOR DEVELOPMENT

Standard



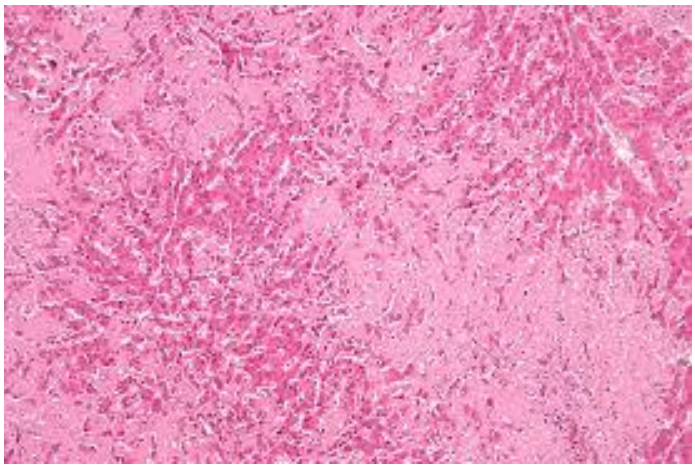
NO TUMOR DEVELOPMENT

Low Dose



NO TUMOR DEVELOPMENT

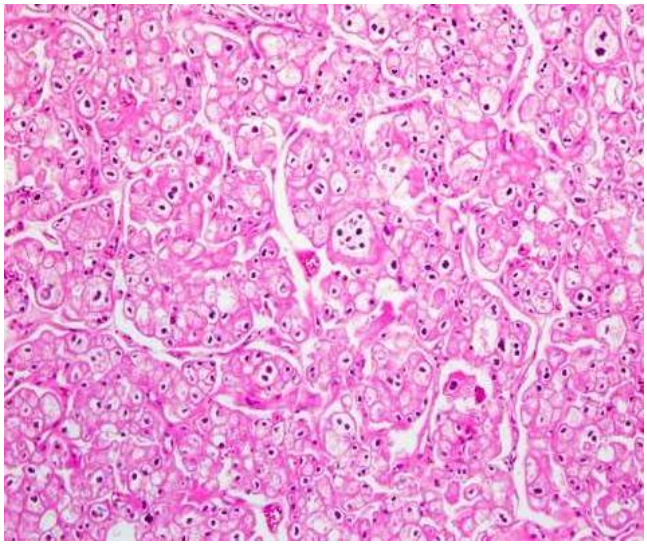
High Dose



NO TUMOR DEVELOPMENT

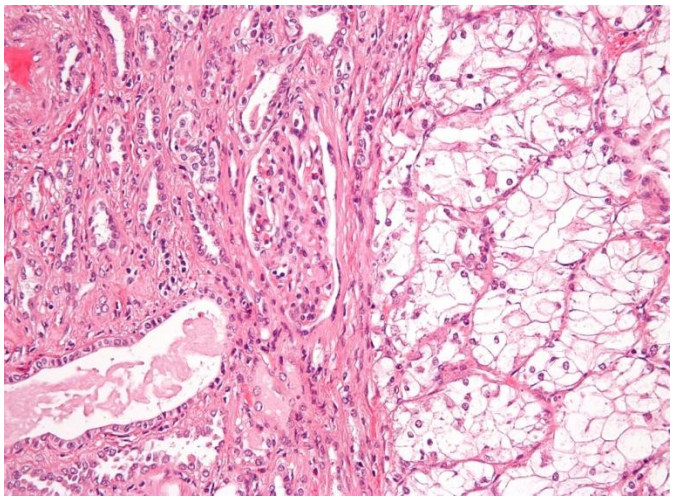
HISTOPATHOLOGY OF KIDNEY

Control Group



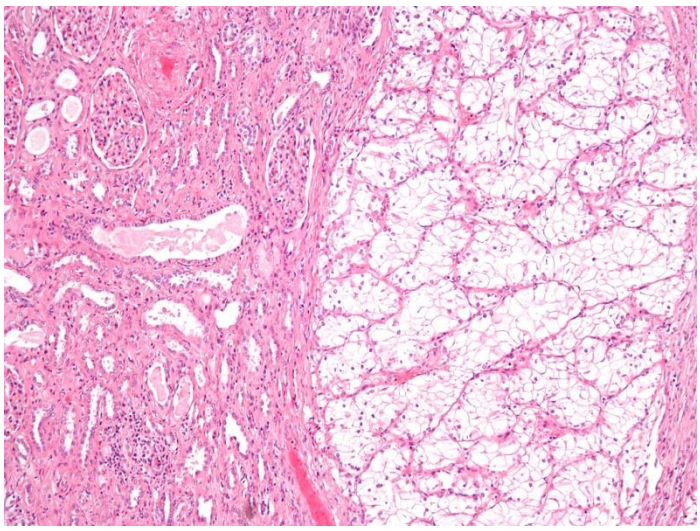
NO TUMOR DEVELOPMENT

Negative Group



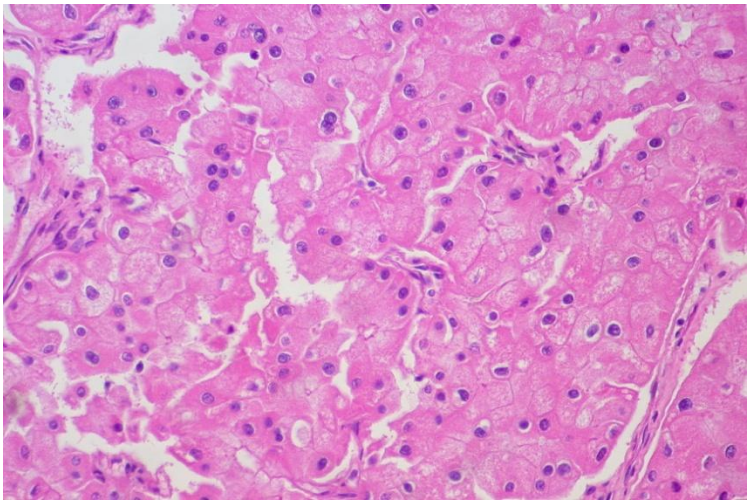
NO TUMOR DEVELOPMENT

Standard Group



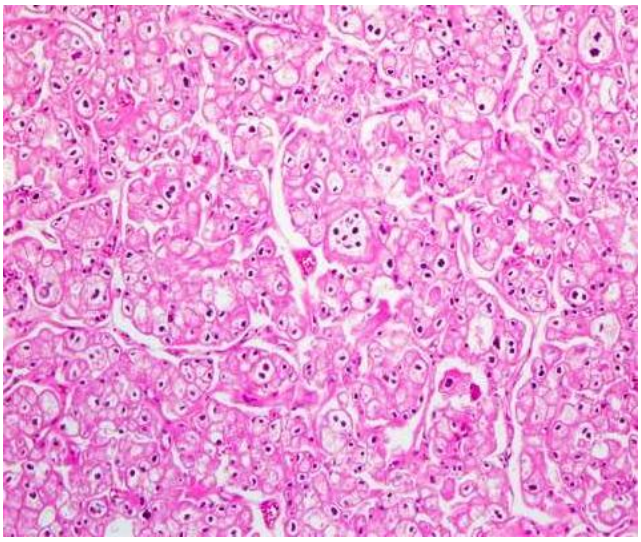
NO TUMOR DEVELOPMENT

Low dose



NO TUMOR DEVELOPMENT

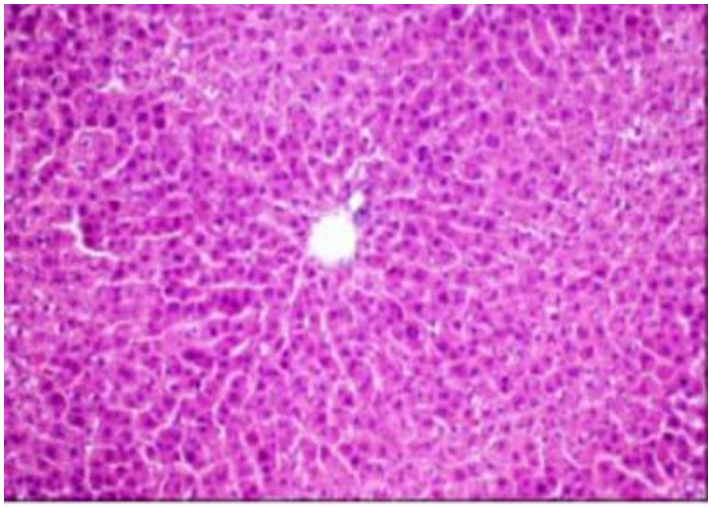
High Dose



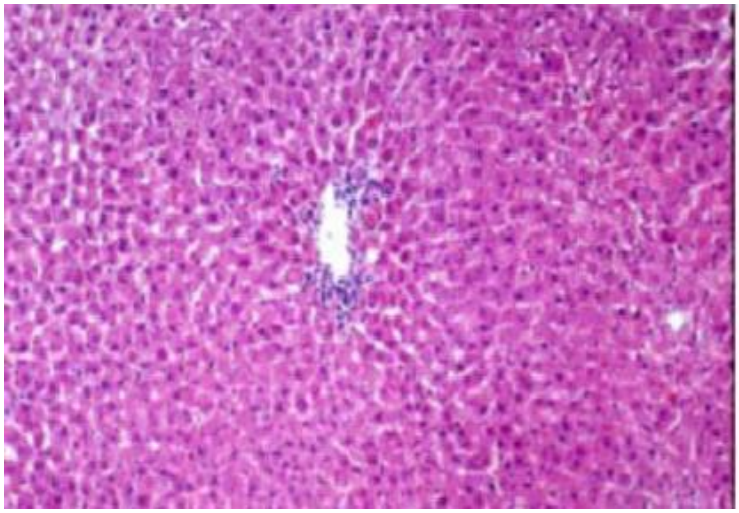
NO TUMOR DEVELOPMENT

HISTOPATHOLOGY OF LIVER

Control Group

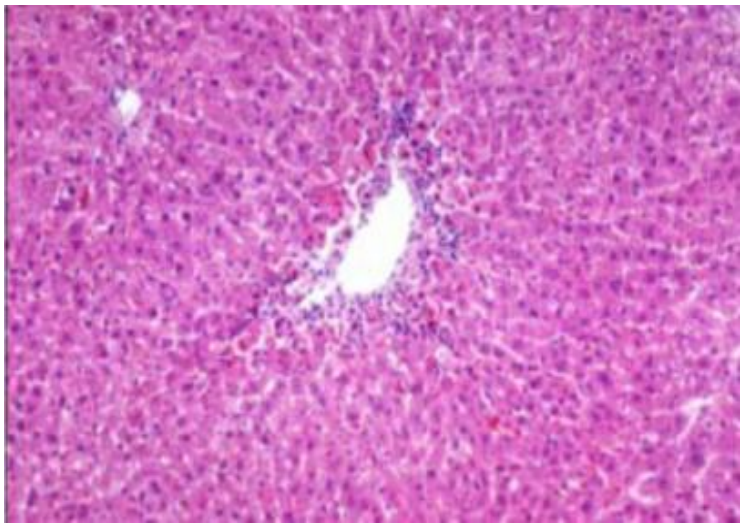


Negative Control



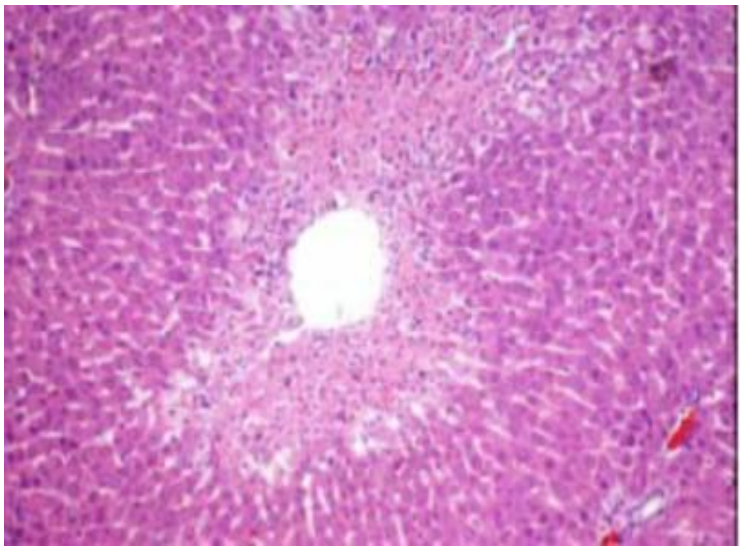
NO TUMOR DEVELOPMENT

Standard



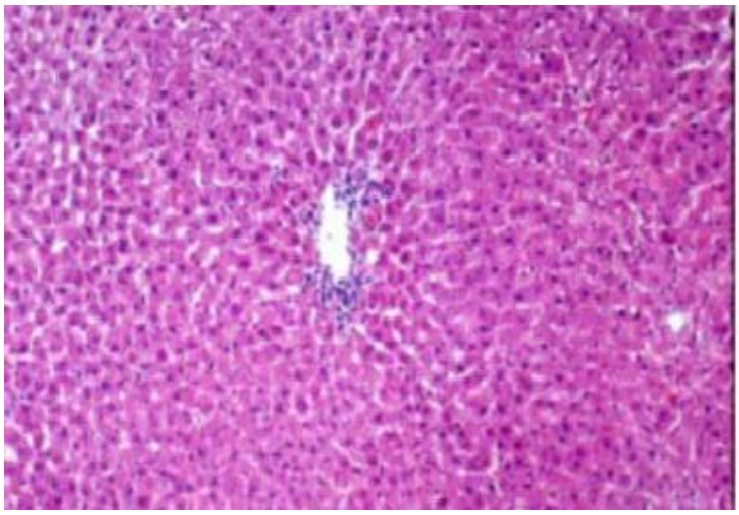
NO TUMOR DEVELOPMENT

Low Dose



NO TUMOR DEVELOPMENT

High Dose



DISCUSSION:

Cancer, also called malignancy, is an abnormal growth of cells[2]. It is one of the most worldwide spread diseases[3]. Cancer appears as a mass or ulcerates. Cancer is a **"great imitator"[4].** It represents a group of several diseases characterized by uncontrolled cell division and growth resulting in tumours, if malignant, may spread to other parts of the body. Neoplastic cells originate from differentiated and specialized cells through a process of regression and de differentiation to a simpler; more primitive stage which divides continuously unlike the normal parent cells. The characteristic properties of neoplasia include; sustained proliferation, evasion of growth suppressors, immortality, anaplasia , continued replication, angiogenesis, reprogramming energy metabolism, invasion, metastasis, and escaping immune destruction[5.]

The multistep process including initiation, promotion, and progression of carcinogenesis is a complicated process that results from excessive production of oxidative radicals, DNA alterations, and lastly the loss of the normal regulatory pathways between cell proliferation, differentiation, and apoptosis[6,7]. Progress made in cancer therapy has not been sufficient to minimize the annual death rates. The currently used conventional anticancer drugs are also toxic to normal cells in addition to being toxic to cancer cells[8]. Hence, there is a greater need for new effective and safe strategies in cancer control and therapy. Prevention is the most practical strategy to control occurrence and spread of cancer. Cancer chemoprevention aims to stop or reverse the development and progression of precancerous cells through the use of non-cytotoxic nutrients and/or pharmacologically active agents. It is also important to provide various cancer chemopreventive natural agents with different specific molecular and cellular targets, which act through multiple mechanisms[9]

> Breast cancer is a malignant tumor that starts in the cells of the breast. A malignant tumor is capable of invading surrounding tissues or spreading or metastasizing to distant areas of the body. The disease occurs almost entirely in women, but men can get it, too[23, 24].

Endocrine therapy targets estrogen receptor-positive breast cancers and represents the most effective treatment for them. In the last four decades, several hormonal agents have been used in palliative settings for advanced cancer and as adjuvant therapy to prevent the recurrence. However, there are several proposed molecular mechanisms that explain endocrine resistance in a large proportion of patients, which limit the effectiveness of endocrine treatments[28, 29,30] .

7,12-Dimethylbenz[a]anthracene (DMBA) is an immunosuppressor and a powerful organ-specific laboratory carcinogen. DMBA serves as a tumor initiator[107]. Tumor promotion can be induced with treatments of 12-O-tetradecanoylphorbol-13-acetate (TPA) in some models of two-stage carcinogenesis[108].

ASVERADO is the herbal product with the combination of Ashwagandha, Aloe vera, Avocado. It consists of tumour suppressing constituent ß-carotene . This constituent act on BRAC 1, BRAC 2 and p53 receptor which play major role in suppressing mammary gland carcinogenesis.

BRCA1 and BRCA2 are normally expressed in the cells of breast and other tissue, where they help repair damaged DNA, or destroy cells if DNA cannot be repaired. They are involved in the repair of chromosomal damage with an important role in the error-free repair of DNA double-strand breaks.[109,110] If BRCA1 or BRCA2 itself is damaged by a BRCA mutation, damaged DNA is not repaired properly, and this increases the risk for breast cancer[111,112]. BRCA1 and BRCA2 have been described as "breast cancer susceptibility genes" and "breast cancer susceptibility proteins". The predominate allele has a normal, tumor suppressive function whereas high penetrance mutations in these genes cause a loss of tumor suppressive function which correlates with an increased risk of breast cancer[113].

This research was aimed to evaluate the both in-vitro studies using Breast cancer cell line (MCF-7) & in-vivo studies based on the ethanolic extract of ASVERADO for its anti-neoplasttic activity against 7,12-dimethylbenzeneanthracene (DMBA) induced Mammary gland carcinoma in female Wistar rats.

The mammary gland is highly sensitive to oxidative damage because of its high oxygen consumption. Thus, oxidative biomarkers such as superoxide dismutase (SOD), catalase (CAT), gl), lipid peroxidase (LPO) are analysed to study the severity of the disease and effect of the drug on it. Level of SOD, CAT, were found to be decreased in affected rats and LPO was observed to be high in its concentration when compared to normal rats. The rats those were treated with ASVERADO were observed to possess antioxidant activity showing the level of above mentioned oxidative biomarkers similar to normal rats.

SUMMARY:

The present study was conducted for evaluation of ameliorating potential in terms of anticancerous and antioxidative properties of herbal ethanolic extract of ASVERADO from fruit of **Persea Americana Mill,** leaves of ***Aloe vera (L.) Burm.f., &*** Root of *Withania Somnifera* on DMBA induced mammary tumorigenesis in Wistar rats.

The preliminary phytochemical studies on ASVERADO discovered the presence of various phytoconstituents such as alkaloid, carbohydrates, glycosides, steroid, proteins, flavonoids and saponin.

This research was aimed to evaluate the both in-vitro studies using Breast cancer cell line (MCF-7) & in-vivo studies based on the ethanolic extract of ASVERADO for its anti-neoplasttic activity against 7,12-dimethylbenzeneanthracene (DMBA) induced Mammary gland carcinoma in female Wistar rats.

The biochemical parameters like Blood Sugar level, Hb count,Albumine, RBC count,PCV, MCV, MCH,MCHC,Total count, Polymorph, Lymphocytes,Eosinophils,Globulin and Total Protein Binding values showed increase in the ASVERADO treated rats when to compared to DMBA treated rats.

The antioxidants defense in the mammary gland tissues were estimated in terms of SOD, CAT, LPO and ASVERDO increased the antioxidant levels at respective dose leading to reduce oxidative stress. This clearly indicates the potential of the extract to delay the generation of free radicals that cause carcinogenesis.

The histopathology of DMBA treated rats showed increased in tumor cells. Whereas the control and ASVERADO treated rats showed normal level of tumor cells.

CONCLUSION:

The present study demonstrated that DMBA induced mammary gland carcinoma in Wistar rats. Ethanolic extract of ASVERADO from fruit of **Persea Americana Mill,** leaves of ***Aloe vera (L.) Burm.f., &*** Root of *Withania Somnifera* which helps in suppressing tumor cells. The anti-oxidant has potential of delaying the generation of free radicals that cause carcinogenesis.

Further studies are expected on the isolated components of the extract in order to understand the exact mechanism of its action and also studies at cellular level changes by more sophisticated methods for investigation.

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ANNEXURE 1

INSTITUTE OF HERBAL SCIENCE
PLANT ANATOMY RESEARCH CENTRE

Prof.**P Jayaraman, Ph.D**
Director
Retd, Professor, Presidency College Chennai-5

AUTHENTICATION CERTIFICATE

Based upon the Organoleptic /macroscopic /~~microscopic~~ examination of fresh /~~market~~ sample, it is certified that the specimen given by Khyathi. D. Sanghvi, II M. Pharm. C L Baid Metha College of Pharmacy, Thoraipakkam, Chennai. 97 is identified as below:

Binomial: Aloe Vera (L.) Burm. f.

Family: Liliaceae

Synonym(s): Aloe perfoliata L.

Regional names: Sans. Grita-kumari

Reg.No of the certificate: PARC/2017/1431

References: Nair, N.C & Henry, A.N. Flora of TamilNadu, India I: .1983.

Henry, A.N. *et al.* Ibid. II: .1987.

Ibid. III: p.37 .1989.

Ed:S.P.Ambasta,
The Useful Plants of India,
CSIR- Publication, 1986.

Date: 27-04-20

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ANNEXURE 2

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AUTHENTICATION CERTIFICATE

Based upon the Organoleptic /macroscopic /~~microscopic~~ examination of ~~fresh~~ /market Powder sample, it is certified that the specimen given by Mr. Jyothi. D. Sangvi, II M.Pharm. CL Baid Metha College of Pharmacy, Thoraipakkam, Chennai-97 is identified as below:

Binomial: Withania somnifera (L.) Dunal

Family: Solanaceae

Synonym(s): Physalis somnifera L.

Regional names: Sans: Ashwagandha

Reg.No of the certificate: PARC/2017/34.32

References: Nair, N.C & Henry, A.N. Flora of TamilNadu, India I: _____ .1983.

Henry, A.N. *et al.* Ibid. II: p. 118 .1987.

Ibid. ~~HI~~: _____ .1989.

Ed:S.P.Ambasta,
The Useful Plants of India,
CSIR- Publication, 1986.

Date: 27.04.2017

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ANNEXURE 3

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AUTHENTICATION CERTIFICATE

Based upon the Organoleptic ✓ /macroscopic ✓ /~~microscopic~~ examination of ~~fresh~~ /market Fruit sample, it is certified that the specimen given by Khyathi D. Sanghvi, T.M.Pharm. C.L. Baid Metha College of Pharmacy, Thoraipakkam, Chennai-97 is identified as below:

Binomial: Persea americana Mill.

Family: Lauraceae

Synonym(s): P. gratissima Gaertn.f

Regional names: Avocado.

Reg.No of the certificate: PARC/2017/3433

References: Nair, N.C & Henry, A.N. Flora of TamilNadu, India I: .1983.

Henry, A.N. *et al.* Ibid. II: .1987.

Ibid. III: .1989.

Ed:S.P.Ambasta, The Useful Plants of India, CSIR- Publication, 1986. } - P. 442

Date: 27.04.2017

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